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THE REACTION OF FOUNDATION STOCK LINES OF SELKIRK WHEAT TO SOME PHYSIOLOGIC RACES OF WHEAT STEM RUST[1]

G. J. GREEN[2] AND T. JOHNSON[3]

Plant Pathology Laboratory, Winnipeg, Man.

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ABSTRACT

The present foundation stock of Selkirk wheat, derived from the cross (McMurachy × Exchange) × Redman[3], is a composite of 42 lines which inherited their resistance to stem rust, *Puccinia graminis* f. sp. *tritici* Erikss. & Henn., from McMurachy and Redman. Seedling tests with several physiologic races of this rust have shown that, while all the lines have inherited rust resistance from both of these ancestors, they nevertheless differ in rust reaction. All the lines seem to have inherited the McMurachy type of resistance but on the basis of their reaction to certain races they can be separated into two groups, presumably because they have inherited different degrees of resistance from Redman. Both groups are resistant to race 56 at low temperature but one of the groups is susceptible to this race at high temperature, as is McMurachy. The group of lines resistant at high temperature is also resistant to a newly discovered biotype of race 56 which attacks McMurachy and the other group of lines at low temperature. The lines resistant to race 56 and its biotype appear to have inherited more resistance from Redman than the other lines. Other tests indicate that, in the Selkirk lines, resistance to some races (e.g. 17, 29, 48, 15B) is derived from McMurachy but resistance to other races (e.g. 48A, 139) is derived from Redman.

INTRODUCTION

Many wheat varieties produced by hybridization are not pure lines but composites of phenotypically similar lines derived from a common origin. The present foundation stock of the wheat variety Selkirk is a composite of 42 lines originating from the cross (McMurachy × Exchange) × Redman[3]. These lines were selected, mostly in field tests, on the basis of their resistance to race 15B and other stem rust races prevalent in North America.

[1] Contribution No. 1421 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Plant Pathologist.

[3] Officer-in-Charge.

The realization of the composite nature of Selkirk prompted a study of the seedling reaction of the lines to several physiologic races of stem rust. The object of these studies was to determine whether the two stem-rust resistant ancestors of Selkirk, McMurachy and Redman, had contributed resistance in the same degree or in different degrees to different lines. It was assumed at the beginning of the study that all the lines contained rust resistance derived from McMurachy because the lines were all resistant to race 15B and McMurachy was the only variety in the parentage of Selkirk that was resistant to this race. Since high temperature reduces the effectiveness of the McMurachy resistance most of these studies were performed at two levels of temperature, about 60° F. and 80° F.

By using certain races that were virulent to McMurachy and others non-virulent to it and, similarly, by using races virulent and non-virulent to Redman, it was hoped that some light could be thrown on the type or types of resistance possessed by the different lines of Selkirk.

MATERIALS AND METHODS

In this investigation, 42 lines, comprising the foundation stock of Selkirk wheat, were studied individually. This variety, derived from the cross (McMurachy × Exchange) × Redman[3], inherited its stem rust resistance from McMurachy and Redman since Exchange is susceptible to many common races (e.g. 15B, 56, 139). The McMurachy resistance has been found to be inherited as a single recessive factor (5) and there can be little doubt that all Selkirk lines possess this type of resistance. Redman has a complex ancestry; it is derived from the cross Regent × Canus. Regent is derived from the cross H44-24 × Reward and Canus from the cross Marquis × Kanred. Reward is a derivative of the cross Marquis × Prelude. Since Prelude is susceptible to many races (4), Redman could conceivably have inherited resistance genes from H44-24, or from Kanred, or from Marquis, or from all of these. However, there is no evidence of the Kanred immunity gene in any of the Selkirk lines. This gene is readily identified as it confers immunity to certain races.

The rust races employed have been isolated from Canadian rust collections obtained in the last few years. They were selected for use in this study because of the infection types they produce on McMurachy and Redman. The culture of race 15B is representative of the commonly occurring form of this race which for several years has constituted most of the stem rust in Canada. Races 17 and 29 have been isolated from Canadian rust collections for many years. Redman is susceptible in the seedling stage to these three races, whereas McMurachy is resistant to them at low temperature but susceptible at high temperature. Redman and McMurachy are resistant to race 48 at low temperature but become more susceptible at high temperature. At low temperature neither variety is attacked by race 56 but McMurachy becomes susceptible at high temperature. Races 48A, 56A and 139 attack McMurachy but not Redman. Of these races 48A is of interest because it is evidently a newcomer to Canada, first isolated from plants of McMurachy that were severely rusted by it in several of the Uniform Rust Nurseries in Eastern Canada in 1954. As Selkirk was virtually free from rust in the same rust nurseries it was evident

that some type of rust resistance was operative in Selkirk that was not derived from McMurachy. Race 56A also requires some comment as its existence has not been reported hitherto. It was discovered in a collection of rust taken from Little Club wheat at Normandin, Que., September 20, 1954, and has not been found in any other rust collection. It is not distinguishable from other cultures of race 56 on the standard differential hosts, but differs by producing a 4 type of infection on McMurachy instead of the 0 or 1 type characteristic of race 56. It may possibly be a mutant of race 56 with pathogenicity towards McMurachy sufficiently increased to permit it to overcome the McMurachy type of resistance at a lower temperature than is possible for other known specimens of that race.

The method of inoculation employed and a description of stem rust infection types have been recorded previously (3). The tests were carried out with seedlings grown in the greenhouse during the fall of 1954 and the spring of 1955. Prior to inoculation they were kept in a greenhouse at ordinary fluctuating temperatures (mean 65°-70° F.). After inoculation the seedlings were placed in greenhouses with temperatures controlled at about 60° and 80° F., excepting a few trials with races 139 and 56A which were carried out at ordinary temperatures.

EXPERIMENTAL

All the lines of Selkirk were resistant to race 56 at relatively low greenhouse temperatures. When tested at high temperature 22 lines were resistant (displaying type 1 infection), 16 were susceptible (type 4 infection) while 4 segregated to produce both types. Since other tests had shown that all the lines were resistant to race 15B at low and ordinary temperatures it seemed evident that they all possessed rust resistance derived from McMurachy, the 15B-resistant progenitor. If all the lines also possessed resistance derived from Redman, none of them should have been susceptible to race 56 at the higher temperature, since Redman was resistant at that temperature level. It seemed possible, therefore, that the lines that failed to show resistance at the high temperature did not possess the Redman type of resistance. To test this hypothesis all the lines were inoculated with race 139 at ordinary greenhouse temperature. This race was chosen because it distinguishes clearly between the Redman type of resistance which is effective against race 139 and the McMurachy type which is not. In this test, all 16 lines that had been susceptible (4 type of infection) to race 56 at high temperature were moderately resistant (2+ type of infection) whereas the lines that had been resistant to race 56 at high temperature were highly resistant (1 type of infection). The first-mentioned lines, showing 2+ type of infection, therefore did not agree with the McMurachy parent which shows a 4 type of infection to race 139. Hence it seems clear that these lines possess some resistance in addition to that of McMurachy. This additional resistance is presumbly derived from Redman.

It was evident from these tests that the Selkirk lines fell into two classes distinguishable by race 56 at high temperature and race 139 at low temperature. Four lines from each class were selected for further trials and were tested to races, 15B, 17, 29, 48, 48A, 56, 56A and 139. Results of the tests are presented in Table 1.

TABLE 1.—INFECTION TYPES PRODUCED BY 8 WHEAT STEM RUST RACES AT HIGH (80° F.) AND LOW (60° F.) TEMPERATURES ON SEEDLING LEAVES OF 8 LINES OF SELKIRK WHEAT AND ITS ANCESTRAL VARIETIES REDMAN AND MCMURACHY

Race	Temp.	Selkirk lines								Redman	McMurachy
		4	11	20	31	6	15	32	45		
15B	High	4−	4−	X	4−	4−	4−	3+	3±	4	4
	Low	1=	0;	0;	0;	0;	0;	0;	0;	4	0;
17	High	X−	X	X−	X−	X+	X+	X−	X−	4	4
	Low	0;	0;	0;	0;	0;	0;	0;	0;	4	0;
29	High	X+	X	X+	X	X	X	X	X	4	4
	Low	0;	0;	0;	0;	0;	0;	0;	0;	4	0;
48	High	2−	2−	2−	2−	2−	2−	2−	2−	3±	X=
	Low	0;	0;	0;	0;	0;	0;	0;	0;	2	0;
48A	High	2±	2±	2±	2±	2±	2±	2±	2±	3	4
	Low	2+	2	2	2	2	2	2	2	2	4
56	High	0;	0;	0;	0;	3	3−	3−	3−	1	3−
	Low	0;	0;	0;	0;	0;	0;	0;	0;	1=	0;
56A	High	−	−	−	−	−	−	−	−	−	−
	Low	0;	0;	0;	0;	3±	3±	3+	3±	0;	3±
139	High	1−	1−	1−	1−	4−	4−	4−	3+	1−	4−
	Low	1=	1=	1=	1=	2	2	2	2	1=	4

Note: "0;" and "1" signify high resistance; "2" moderate resistance; "3" moderate susceptibility, and "4" full susceptibility. "X" indicates a reaction on the borderline of resistance and susceptibility. "=" and "−" indicate that the size of rust pustules is below normal for the type, and "+" that pustules are somewhat larger than normal.

In the test with race 29, at low temperature, the eight Selkirk lines reacted like McMurachy, displaying only minute flecks. Therefore it appeared that Selkirk had derived its resistance to this race from McMurachy rather than from Redman which was susceptible to it.

In the test with race 48, also, the Selkirk lines were identical in reaction with McMurachy. In the test with race 48A, they behaved like Redman, which is moderately resistant but differed strikingly from McMurachy which is susceptible. Therefore resistance appears to be inherited from Redman. This resistance is very similar, as judged by the infection type, to the resistance of Marquis to this same race. Consequently, resistance to this race is not necessarily traceable through Redman to H44-24 but may possibly be traceable to Marquis through Reward.

All of the Selkirk lines were tested for their reaction to race 56A which differs from race 56 in its ability to attack McMurachy. Those lines that were susceptible to ordinary race 56 only at high temperatures were susceptible to the new type of race 56 at low temperatures; that is, they reacted like McMurachy. The lines that were resistant to ordinary race 56 at high temperatures were resistant to the new type of race 56. This race therefore distinguishes, at relatively low temperatures, between the two groups of lines and does so more effectively than race 139.

DISCUSSION

It has been demonstrated that the reaction of Selkirk foundation lines to certain stem rust races provides some indication as to whether resistance was derived predominantly from McMurachy or from Redman. It is clear that all the Selkirk lines contain resistance factors derived from both these ancestors, and consequently these lines can resist certain races that attack McMurachy, such as races 48A and 139 and certain races that attack Redman such as race 15B. It seems clear, too, that no large number of resistance factors, at least factors detectable by the races so far studied, are responsible for the differences between the lines; otherwise the Selkirk lines would not fall into merely two distinct classes. It is not clear how many factors derived from each of the two ancestors, McMurachy and Redman, are concerned in the rust reaction of Selkirk. The results obtained seem most readily understood by assuming that McMurachy contributed a single pair of genes to each line but that Redman contributed at least two pairs of genes to the most resistant lines and a smaller number, possibly a single pair of genes, to the more susceptible lines. This seems a reasonable assumption since Redman was the recurrent parent in the crosses that produced Selkirk.

If this suggestion is tentatively accepted, the origin of the genes contributed by Redman still remains unexplained. The reaction of Selkirk lines to races such as 48A and 139 is the same as that of Marquis, and consequently it is not unlikely that the factors conditioning resistance to these races are derived from Marquis, since this variety occurs repeatedly in the ancestry of Selkirk. There is no clear evidence for the presence of genes from H44-24 although it is quite possible that the additional resistance that some lines show to race 56 at high temperatures is derived from this progenitor. This resistance could scarcely have come from Marquis or from Kanred as both are susceptible to race 56.

It is not easy to identify the Selkirk factors definitely with any previously described. Goulden, Neatby and Welsh (1) stated that in the cross H44-24 × Marquis there was a difference between the parents of two pairs of factors for seedling resistance to race 36 and probably also for resistance to race 21. Their statement that F_4 lines breeding true for reactions to these races gave the same reactions as Marquis to a group of races including race 14, to which Marquis is resistant, indicates that one of the factors they were dealing with came from Marquis.

Neatby (2), in a study of the seedling reaction of hybrid lines from the cross Marquis × H44-24 to 15 physiologic races, postulated the operation of two sets of factors, each set probably consisting of a single pair of genes. The genes postulated do not appear to be definitely traceable to either of the parents.

It seems evident from the results reported in the present paper that in the production of rust-resistant varieties composed of many different lines it is highly desirable to study the reaction of these lines to as many rust races as possible. Through a knowledge of reaction of the lines to specific races it is possible to re-group the lines in such a way as to eliminate those susceptible to particular rust races. This manoeuvre may be of importance as a countermeasure to the increasing prevalence of races that can

attack some but not all of the lines that constitute a variety. It is evident, too, that a close study of the reaction of the various lines to specific races provides some indication of the ancestral source of the resistance factors contained in a given line.

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THE EFFECT OF FUNGICIDES ON SEEDLING DISEASES OF LEGUMES AND GRASSES IN SASKATCHEWAN[1]

H. W. MEAD[2]

Plant Pathology Laboratory, Saskatoon, Sask.

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ABSTRACT

Control of seedling blight of alfalfa, clovers, and grasses by treatment of the seed with various fungicides was studied in laboratory, greenhouse and field experiments. Germination on blotters was not improved by seed treatment. In sterile soil inoculated with *Fusarium culmorum*, treated seed of alfalfa, red clover and crested wheat grass grew better than untreated seed. The results of other greenhouse experiments conducted in natural soil showed that the effects of seed treatment varied with different samples of seed and soil. In most of the experiments, seed treatment considerably improved the stand of legumes but made little change in the growth of grasses. Data from field experiments also indicated a wide variation in the effect of fungicides applied to the seed. Alfalfa and red clover benefited more than sweet clover; grasses in all cases responded less favourably to seed treatment than legumes and dosage was more critical in these crops. The newer fungicides, based on captan, were less toxic than others and caused improvement in stand of the legumes and grasses. Seed treatment did not control post-emergence blighting.

INTRODUCTION

Farmers in Saskatchewan have experienced difficulty in obtaining uniform stands of legumes and grasses. Among the many causes of losses in stand is seedling blight caused by soil- and seed-borne fungi, which include *Pythium debaryanum* Hesse, *Rhizoctonia solani* Kuehn, *Fusarium acuminatum* Ell. & Ev., *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Sm.) Sacc., and *Ascochyta imperfecta* Peck.

The main purpose of the present study was to obtain information on methods of control of seedling diseases of legumes and grasses in Saskatchewan by means of fungicides applied to the seed. This subject has been studied extensively in the United States and the results have been reviewed recently by Kreitlow, Garber, and Robinson (1). These authors concluded from their own experiments that results from treatment of forage crop seeds with fungicides may be advantageous in some cases.

This conclusion is supported by the fact that the writer of the present paper has shown (2) that growth of *Ascochyta imperfecta* from alfalfa seed plated on agar was prevented by four fungicides.

MATERIALS AND METHODS

Commercial seed of alfalfa, clovers and grasses, grown in Saskatchewan, was used in these investigations. This seed represented the varieties recommended for this province and it included samples of good and poor seed. The fungicides which were used included those currently offered for sale and new types offered by chemical companies. These fungicides represented organic mercurials, carbamates, naphthoquinones, chloranil,

[1] Contribution No. 1439 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

[2] Plant Pathologist.

and captan products. Unless otherwise indicated, the seed was treated with an excess of the fungicide dust and the surplus was screened off. One of the organic mercurials, Panogen, was applied as a liquid at the recommended rate.

The investigations included experiments in the laboratory and greenhouse and in field plots. Counts of stand usually were made 10 to 20 days after sowing. In one field experiment the stand was measured by digging and counting the number of plants in a given length of row, when they were about four months old. In another experiment, where weed growth was so dense that seedlings could not be counted, stand was measured with a point sampler. This tool is a wooden frame with stiff wire pins projecting at regular intervals. The frame is dropped at random on the area to be sampled and the stand is estimated by the number of 'hits' of the pins on plants.

EXPERIMENTAL RESULTS

Effect of Fungicides on Germinability of Seeds of Legumes

During germination tests moulds of various kinds occasionally cause rotting of seed and blighting or malformation of seedlings. The effect of nine fungicides on germination of alfalfa, sweet clover and red clover on blotters was studied in the laboratory by growing untreated and treated seed for ten days at 70° F. The number of normal plants was altered slightly by the fungicides. In about one-half of the seed samples there was a reduction in normal germination of the treated portions; of those that were improved by treatment, only one treated lot (Spergon on sweet clover) was significantly better than the check. These results are given in Table 1.

TABLE 1.—PERCENTAGE OF HEALTHY SEEDLINGS OF ALFALFA, SWEET CLOVER, RED CLOVER, AND ALSIKE CLOVER, TREATED WITH DIFFERENT FUNGICIDES AND GROWN ON BLOTTERS AT 70° F. FOR TEN DAYS

Treatment	Rate of treatment (percentage weight of seed)	Alfalfa	Sweet clover	Red clover	Alsike clover
Arasan	0.50	72	31	48	71
Ceresan	0.05	71	26	50	61
Ceresan M	0.05	56	33	53	69
Leytosan	0.05	52	33	51	68
Spergon	0.30	58	37	50	71
Fungicide 604[1]	0.20	55	26	49	64
Phygon	0.20	62	30	58	65
Thiram[2]	0.20	60	24	50	71
Ferbam	0.50	63	31	53	67
Check	—	68	25	53	67

L.S.D. 5 per cent, 7.9.
1 per cent, 11.2.

[1] Fungicide 604—U.S. Rubber Co.
[2] Thiram—Naugatuck Chemicals.

Effect of Fungicides on Seedling Blight of Legumes and Grasses Under Greenhouse Conditions

Seed of legumes and grasses was sown in wooden flats or in large beds filled with soil. The seed was scattered in shallow depressions and covered lightly, and the soil was kept moist by light watering. The temperature of the greenhouse was 70° to 75° F. In one set of experiments sterilized soil was inoculated with *Fusarium culmorum*; in other experiments, soil known to be infested with seedling blight fungi was used.

Counts of healthy seedlings made 10 to 12 days after seeding showed that Arasan increased the stand of both good and poor* seed of alfalfa, sweet clover and red clover, in soil inoculated with *F. culmorum*. The increases were about equal in both grades of seed. There was little change in the growth of brome grass and good crested wheat grass after treatment, but poor seed of crested wheat grew much better when treated. These comparative results with legumes and grasses were unexpected, because *F. culmorum* usually is more parasitic on grasses than on legumes. The results are shown in Table 2.

* *Good seed*—80 to 100 per cent germination.
Poor seed—below 80 per cent germination.

TABLE 2.—PERCENTAGE OF HEALTHY SEEDLINGS OF LEGUMES AND GRASSES TREATED WITH ARASAN AND GROWN IN STERILIZED SOIL INOCULATED WITH *Fusarium culmorum*

Treatment	Alfalfa		Sweet clover		Red clover		Brome		Crested wheat	
	Good	Poor	Good	Poor	Good	Poor	Good	Poor	Good	Poor
Arasan	52[1]	50	83	48	68[2]	29	89	47	77	43[2]
Check	38	40	75	33	42	21	86	47	80	32

[1] Significant to 5 per cent point.
[2] Significant to 1 per cent point.

TABLE 3.—PERCENTAGE OF HEALTHY SEEDLINGS OF ALFALFA, SWEET CLOVER, BROME GRASS AND CRESTED WHEAT GRASS, TREATED WITH FUNGICIDES AND GROWN IN NATURAL SOIL IN THE GREENHOUSE FOR 10 DAYS AT 65° TO 75° F.

Treatment	Alfalfa	Sweet clover	Brome	Crested wheat
Arasan	51	51	78	68
Semesan	53	43	76	68
Panogen	68	59	71	63
Phygon	48	45	74	64
Spergon	37	35	—	—
Check	40	42	79	69

L.S.D. 5 per cent, 9.5.
1 per cent, 12.8.

TABLE 4.—A SUMMARY OF FIVE GREENHOUSE EXPERIMENTS SHOWING THE EFFECT OF FUNGICIDES ON THE STAND OF LEGUMES AND GRASSES

(+ = increase; − = decrease in stand; 0 = no change, when compared with the check)

	Alfalfa					Sweet clover					Red clover			
LEGUMES														
	Experiment					Experiment					Experiment			
Treatment:	1	2	3	4	5	1	2	3	4	5	1	2	4	5
Ceresan		+[2]					+[2]					+		
Semesan		+[2]	+[1]				+[2]					+		
Panogen			+[2]					+[2]						
Arasan	0	+[1]	+[1]	+[1]		−	+[1]	+[1]	+	+	+[2]	+	+	+
Phygon	+[2]	+	+		−	−	+[2]	+		+	−	+		−
Spergon	−	+	−		+	−	+	−		−	+[2]	0		+
Ferbam	+[2]					−					0			
Orthocide 75					+					+				+
Orthocide 406					+					+				+

	Brome grass				Crested wheat grass			
GRASSES								
	Experiment				Experiment			
Treatment:	2	3	4	5	2	3	4	5
Ceresan	−				+			
Semesan	−	−			+	−		
Panogen		−				−		
Arasan	+	−	+	−	+	−	−	−
Phygon	−	−		−	+	−		+
Spergon	−				+			
Orthocide 75				0				+

[1] Significant at 5 per cent level.
[2] Significant at 1 per cent level.

The results of other greenhouse experiments conducted in natural soil show that the effects of seed treatment varied with different samples of seed and soil. In most of the experiments the stand of legumes was improved considerably by seed treatment while there was little change in the growth of the grasses. Data from a typical experiment are given in Table 3. In this experiment 10 samples each of alfalfa, sweet clover, brome and crested wheat were treated and sown in natural soil.

In other greenhouse experiments, the mercury fungicides improved the stand of alfalfa and clovers, but usually injured brome and crested wheat. Dosage was found to be critical when grass seed was treated with these fungicides. Arasan caused increases in 12 out of 14 trials on legume seed, and 6 of these results were significantly greater than the checks, but this fungicide was much less effective on grasses. Variable results were obtained with Phygon and Spergon, the effects ranging from significant increase to significant decrease. Two captan preparations, Orthocide 75 and Orthocide 406, have been used for one year only; both have been beneficial to alfalfa and clovers and have either slightly improved or caused no change to grass stands. A summary of the greenhouse experiments is given in Table 4.

Effect of Fungicides on Stand of Legumes and Grasses in Field Experiments

Experiments were conducted in field plots at Saskatoon and at Melfort, two areas distinct in soil type and rainfall. At Saskatoon the soil is dark brown loam and the average rainfall from April to October is 10 to 11 inches; at Melfort the soil is black loam and the average rainfall for the same period is 12 to 13 inches.

An experiment at Melfort was designed to show what effect Arasan had on decay of sweet clover seed and infection of the seedlings in the field. To permit recovery of seeds and seedlings the seed was sown in shallow troughs made of zinc screening. The troughs were placed in shallow depressions in the soil in such a way that when a thin layer of soil was placed in the troughs, the seed sown and then covered lightly with soil, the top layer was level with the surface of the plot. Seeding was done by hand at two rates: 50 and 100 seeds per foot. When most of the plants were at the three-leaf stage, the troughs were lifted and the seedlings and ungerminated seeds were separated from the soil by gentle washing.

Data on the number of healthy and diseased seedlings, and decayed and hard seeds, were obtained. These data showed that treatment with Arasan increased the number of healthy seedlings and the total number of seedlings and reduced the amount of seed decay, but did not prevent lesioning of young seedlings by soil fungi. The increases in total stand and healthy seedlings were statistically significant despite considerable variation between the seven replicates. Rate of seeding and incidence of seedling blight were not closely related; more blighting of seedlings occurred in the thickly-seeded, untreated plots than in those with normal seeding, but the effect of seed treatment on seed rotting was similar at the two rates. A summary of this experiment is given in Table 5.

TABLE 5.—PERCENTAGE OF HEALTHY AND DISEASED SEEDLINGS AND DECAYED SEEDS OF SWEET CLOVER, TREATED WITH ARASAN AND GROWN IN THE FIELD AT MELFORT, SASKATCHEWAN

	Normal seeding		Heavy seeding	
	Arasan	Check	Arasan	Check
Healthy seedlings	71	65	79[1]	68
Diseased seedlings	11	5	3	4
Decayed seeds	1[2]	17	1	11
Hard seeds	17	13	17	17

[1] Significant at 5 per cent level compared with check.
[2] Significant at 1 per cent level compared with check.

Five other field experiments were conducted at Melfort and Saskatoon, in which the seed was sown with a V-belt seeder in rows at a uniform rate and depth. In the first three experiments, the seedlings were counted at the three-leaf stage; in Experiment 4, all of the plants in a 4-foot length of each row were dug and counted when they were about four months old. The data for Experiment 5 were obtained by measuring the stand with a point sampler about five months after seeding. The results of these experiments are summarized in Table 6.

An examination of the results shows that alfalfa and red clover benefited more from seed treatment in field experiments than sweet clover. In the case of the latter crop, decreases in stand were almost as frequent as increases. There was little difference in effect of the fungicides on the legumes; however Semesan and the captan products were slightly better than the others. A direct comparison of the results with alfalfa and clovers at Melfort and Saskatoon cannot be made because different experiments were conducted at each location, but it is apparent that the effects of treatment were variable at both places.

Throughout the course of these studies on seed treatment there was always less improvement of grasses than of legumes, and there were many instances of toxicity, especially by organic mercury fungicides.

From a practical standpoint, the non-mercury fungidides are the best for legume seed because they are the least toxic to nitrifying bacteria. Among the newer fungicides, the captan compounds appear to be effective on both legumes and grasses.

CONCLUSIONS

Results obtained from laboratory, greenhouse and field experiments indicated that the response of legume and grass seed to treatment varied considerably because of differences in seed samples and soil conditions. Because such variations in seed and soil do occur, no consistent response to treatment should be expected in these crops. There is sufficient evidence from the experiments described in this paper, however, to show that treatment of forage crop seed might be beneficial on some farms. The cost of

TABLE 6.—A SUMMARY OF FIVE FIELD EXPERIMENTS SHOWING THE EFFECT OF FUNGICIDES ON THE STAND OF LEGUMES AND GRASSES

(+ = increase; − = decrease; 0 = no change, as compared with the check.
Experiments 1 and 3 at Melfort; 2, 4 and 5 at Saskatoon)

	Alfalfa					Sweet clover					Red clover				
LEGUMES	Experiment					Experiment					Experiment				
Treatment:	1	2	3	4	5	1	2	3	4	5	1	3	4	5	
Semesan		+[2]		+[2]			+		+[1]				+[2]		
Panogen		+[2]	−				+[1]	−				−			
Arasan	+	+[1]	+	+	+	−	+	−	+[1]	−	−	−	+[2]	+	
Phygon		+	+	+[2]	+		−	−	−	+		+	+[2]	+	
Spergon			+[1]	+[2]	+			0	−	+		+	+[2]	+	
Thiram			+[1]					−				+			
Orthocide 406				+[2]	+				+[2]	+[1]			+[2]	+	
Orthocide 75					+					+[1]				+[1]	

	Brome grass			Crested wheat grass			Intermediate wheat grass
GRASSES	Experiment			Experiment			Experiment
Treatment:	1	3	5	1	3	5	3
Panogen		−			−		−
Agrox C			−			−	
Arasan	+	+[1]	−	+	+	+	+
Phygon		+	−		−	+	0
Spergon		+[1]			−		+
Thiram		+			−		+
Orthocide 75			+			+	

[1] Significant at 5 per cent level.
[2] Significant at 1 per cent level.

treatment is low and fungicides with a margin of safety in dosage are available now. The fungicides that do not contain mercury do not injure bacterial inoculum seriously and any injury done is likely to be offset by an abundance of natural inoculum in Saskatchewan soils.

Post-emergence blighting occurred in all of the experiments and apparently was not affected by fungicides applied to the seed.

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CHROMATOGRAPHIC STUDIES ON PROTEOLYTIC BACTERIA IN THEIR RELATIONSHIP TO FLAVOUR DEVELOPMENT IN CHEDDAR CHEESE

A. R. Yates, O. R. Irvine and J. D. Cunningham

Departments of Dairying and Bacteriology, Ontario Agricultural College, Guelph, Ontario

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ABSTRACT

A series of 350 bacterial cultures isolated from cheese factory sources were examined for proteolytic activities when incubated at 13° C. for 21 days. Paper chromatographic studies showed that certain isolates were capable of liberating leucine and other amino acids from rennet-digested skim milk but not from sterile skim milk. Cultures identified as *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus liquefaciens*, *Lactobacillus casei* and *Sarcina lutea* possessed this ability to a greater degree than the remaining isolates. These were added to pasteurized milk and made into Cheddar cheese to determine their cheese flavour stimulating powers. After 3 to 10 months' curing, vats containing *Lactobacillus casei* and *Sarcina lutea* had more flavour and better texture than the control lots. *Streptococcus liquefaciens* produced bitter flavours and weak-bodied cheeses while vats containing added *Streptococcus lactis* and *Streptococcus cremoris* cultures were similar to the controls.

INTRODUCTION

Since the beginning of this century, many investigations have been conducted on the subject of flavour development in Cheddar cheese. None of these has demonstrated clearly the reasons for the lack of flavour development in cheese made from pasteurized milk as compared to that made from raw milk. However, a number of recent reports (2, 3, 5, 7, 9) substantiate the theory that protein degradation as indicated by the liberation of amino acids during the curing of cheese is closely associated with flavour development.

It is generally accepted that one of the chief reasons for differences in flavour between raw and pasteurized milk cheeses is the variation in the microflora of the two types of cheese, pasteurization greatly reducing the numbers of organisms in the milk so treated. More information concerning organisms normally present in raw milk cheese and capable of liberating flavourful nitrogenous constituents may aid in the elucidation of this problem.

MATERIAL AND METHODS

Bacteriological Methods

Employing V-8 Juice Agar and Tryptone Glucose Extract Agar, isolates were obtained from samples of raw milk cheeses, raw curd, raw whey and raw milk from cheese factories in Western Ontario and from cheese instructors in various areas of the province. Standard methods were employed for isolations and discrete colonies representative of the flora were subsequently subcultured. Three hundred and fifty cultures were obtained in this manner. All the isolates were examined for their amino acid-liberating properties by paper chromatography and proteolytic

TABLE 1.—DIFFERENTIAL CHARACTERISTICS OF LACTOBACILLUS SPECIES[1]

Name of organism	Litmus milk[2]	Morphology	Lactate utilization	° C. temp. ranges for growth		
				Min.	Opt.	Max.
"L. casei"	RCA	Rods, squared ends	−	10	30	40
"L. plantarum"	AC	Rods, squared ends	−	10	30	40
"L. brevis"	A	Rods, rounded ends	+	10	30	37

[1] Adaptation by Gray (4) from Bergey (1).
[2] Litmus Milk: *R*—Reduced, *C*—Curd formation, *A*—Acid production.

species were identified using Bergey's Manual (1). "*Lactobacillus*" spp. were tentatively identified by the scheme outlined by Gray (4) from Bergey. This is shown in Table 1.

Chromatographic Analyses

Partition chromatography, employing filter paper as a base material, is now widely used in analysing many materials for amino acids and amines. In this investigation, a simple chromatographic method was used to select a number of cultures of the micro-organisms capable of liberating some of these compounds. Several of the cultures thus identified were later used in cheesemaking experiments.

The cultures under investigation were inoculated into tubes of sterile skim milk and tubes of sterile rennet-digested skim milk. The latter was prepared by allowing commercial rennet added at the rate of 1 ml./1000 ml. of milk, to act on fresh, pasteurized skim milk for a period of 16 hours at 25° C. At the conclusion of this period the tubes of digested milk were autoclaved at 15 lb. p.s.i. for 20 minutes.

After incubation at 13° C. for 21 days, 0.01 ml. aliquots from each tube were spotted 5 in. from the narrow edge of 18 in. × 24 in. sheets of Whatman filter paper. These were developed according to the system outlined by Woiwod (10) using 250 ml. of n-butanol and water (50/50 v/v) + 30 ml. of acetic acid as the solvent. Controls of a 0.01 per cent d-l-leucine solution, uninoculated skim milk and rennet-digested skim milk were included in preparing chromatograms. Leucine control checks were used since Kass (6) reported this amino acid as a possible cheese flavour-producing agent. Other amino acid groups were tentatively identified by Rf values from the leucine control spots.

Cheese Manufacture

Small experimental lots (10 litres) of Cheddar cheese were made in a manner closely simulating commercial methods but using a five-section vat. Cultures indicating leucine liberation were added as a light inoculum consisting of 20 ml. of a 48-hour skim milk culture of the test organism and 1 per cent lactic starter to 25 lb. of pasteurized milk. A second vat was also prepared using a heavy inoculum of the test culture. This consisted of 0.5 per cent lactic starter plus 80-100 ml. of the culture to 25 lb. of pasteurized milk. Experimental cheese were stored at 16° C. for 14 days

TABLE 2.—CHROMATOGRAPHIC RESULTS ON MILK PROTEIN BREAKDOWN BY ISOLATED STRAINS

Strain		Protein breakdown			
		Leucine liberation		Other products	
		Rennet milk	Skim milk	Rennet milk	Skim milk
S. faecalis	No. 252	+	–	0	0
S. liquefaciens	No. 15F	++++	–	I, IV, V, VII, VIII	VI
S. liquefaciens	No. 118	–	–	VI	VI
S. liquefaciens	No. 257	–	–	VI	VI
S. liquefaciens	No. 287	++++	–	I, II, IV, VII, VIII	VI
S. cremoris	No. 44	+++	–	0	0
S. cremoris	No. 93	+	–	0	0
S. cremoris	No. 103	+++	–	0	0
S. cremoris	No. 151	+	–	0	0
S. cremoris	No. 230	++	–	0	0
S. cremoris	No. 242	+	–	0	0
S. cremoris	No. 245	+	–	0	0
S. cremoris	No. 309	–	–	III	0
S. lactis	No. 179	+	–	0	0
S. lactis	No. 180	+++	–	0	VI
S. lactis	No. 183	+++	–	0	0
S. lactis	No. 189	+++	–	0	VI
S. lactis	No. 262	+++	–	III	0
L. casei	No. 77	+++	–	V, VIII	0
L. casei	No. 88	–	–	III	0
L. casei	No. 104	+++	–	0	0
L. casei	No. 144	++++	–	0	0
L. casei	No. 147	+++	–	0	0
Sarcina lutea	No. 15	+++	–	VIII	VI

++++, +++, ++, + = Strong to weak leucine liberation.

I Alanine.
II Aspartic acid.
III Arginine-lysine-histidine group.
IV Glutamine-threonine.
V Glycine-serine group.
VI Peptides.
VII Tyrosine.
VIII Valine-methionine group.

TABLE 3.—COMMERCIAL SCORES AND GRADERS' REMARKS ON CHEESE MADE FROM PASTEURIZED MILK AND CULTURED WITH PROTEOLYTIC BACTERIA

Code	Inoculation	First scoring			Second scoring		
		Age (mo.)	Flavour score	Remarks	Age (mo.)	Flavour score	Remarks
Q1	Control	6	40	Bland, lacks flavour, slightly bitter	9	40	Clean but lacks flavour
Q2	S. lutea 15L[1]	6	40	More mature flavour than Q1	9	40	Slightly bitter, slightly weak
Q3	S. lutea 15H[2]	6	40+	More cheese flavour than Q2	9	41	Nice cheese flavour and odour
X1	Control	4	38	Starter flavour, slightly bitter taste, weak body	9	39	Clean but lacks flavour
X2	S. liquefaciens 15FL	4	39	Slightly bitter taste, more cheese flavour	9	40	Clean, medium flavour
X3	S. liquefaciens 15FH	4	37	Very bitter, very weak body	9	36	Bitter flavour, weak body
X4	S. liquefaciens 287L	4	39	Slightly weak body, passable flavour	9	39	Slightly tallowy, otherwise good
X5	S. liquefaciens 287H	4	35	Very bitter, fruity unclean taste, very weak body	—	—	
T1	Control	5	39	Slightly bitter, lacks flavour			
T2	S. liquefaciens 118L	5	39	More bitter than T1			
T3	S. liquefaciens 118H	5	Below	Very bitter, very weak			
T4	S. lactis 262L	5	40	Nice body, good cheese flavour			
T5	S. lactis 262H	5	40+	More flavour than T4			
S1	Control	5	39				
S2	S. lactis 189L	5	38	Slightly fruity flavour, weak			
S3	S. lactis 189H	5	39+	Swiss flavour, open			
W1	Control	4	39	Maturing, slightly bitter	9½	37	Like bitter limburger, weak body
W2	S. lactis 180L	4	39	Maturing, slightly bitter	9½	39	Slightly unnatural flavour
W3	S. lactis 180H	4	39	Slightly bitter, slightly more flavour than W2	9½	39+	More cheese flavour than W2

U1	Control	3	40	Clean taste, slightly gassy odour	10	39	Fairly clean but lacks flavour
U2	S. cremoris 44L	3	40	Clean, slightly lacking in flavour	10	40	Very slightly foreign flavour, mature
U3	S. cremoris 44H	3	40	Clean, more cheese flavour than U2	10	40	Good, except for slightly bitter, slightly oxidized flavour
U4	L. casei 104L	3	41	Clean and nutty but lacks flavour	10	40+	Very good maturity, slightly foreign flavour
U5	L. casei 104H	3	41	More breakdown and maturity than U4	10	41	Very good maturity
V1	Control	4	40+	Clean, nutty flavour, good texture	10	40	Nice clean flavour
V2	L. casei 144L	4	40	Slightly more flavour and breakdown than V1	10	40+	Excellent flavour and body
V3	L. casei 144H	4	40+	Pronounced cheese flavour, good texture	10	41	Very well matured
V4	L. casei 147L	4	39.5	Very slightly bitter, not too clean	10	41	Cleaner than V3, slightly less cheese flavour
V5	L. casei 147H	4	40	Less cheese flavour than V3	10	41	Cleaner than V3, well matured

[1] L = 20 ml. inoculation + 1per cent lactic starter in 25 lb. of milk.
H = 80-100 ml. inoculation + 0.5 per cent lactic starter in 25 lb. of milk.

and at 10° C. for the remaining time. Grading of the cheese was done by an experienced panel of judges who examined each group without being informed of the treatment given.

RESULTS AND DISCUSSION

Of 350 cultures tested, 24 were found to be capable of protein degradation at 13° C. as detected by paper chromatography. Their action is summarized in Table 2. These cultures included eight strains of *Streptococcus cremoris*; five of *Streptococcus lactis*; five of *Lactobacillus casei*; four of *Streptococcus liquefaciens*; one of *Streptococcus faecalis*, and one of *Sarcina lutea*. These organisms produced a greater proteolysis in rennet-digested milk than in skim milk as indicated by paper chromatography. The production of peptidase by eleven cultures was indicated by the liberation of leucine. One strain of *Sarcina lutea* (No. 15), three of *S. liquefaciens* (No. 15F, 118, 287), three of *S. lactis* (No. 180, 189, 262), one of *S. cremoris* (No. 44), and three of *L. casei* (No. 144, 147, 104) were incorporated into experimental cheese. The latter were scored at 3 to 6 months and again at 9 to 10 months of age. Flavour scores and remarks of the graders are recorded in Table 3. The grader's comments are more indicative of the degree of flavour development than is the flavour score. The latter is an index of quality and of freedom from undesirable characteristics.

Individual incorporations in cheese of one strain of *S. cremoris* and three of *S. lactis* produced no noteworthy improvement in cheese flavour. The three strains of *S. liquefaciens* exhibited the greatest proteolytic activity of any of our isolates. However, they produced a bitterness apparently characteristic of this species (8). In addition, the body of the cheese made with *S. liquefaciens* was extremely weak in the result of the extent of proteolysis.

The cultures of *L. casei* improved the flavour development in the experimental cheese. The improvement was greater with the higher rates of inoculation and was more pronounced at 10 months than at 4 months. The development of Cheddar flavour was somewhat improved in cheese made from milk inoculated with *Sarcina lutea*. This organism, however, is not generally found in significant numbers in Cheddar cheese.

Undesirable flavours were produced by *S. liquefaciens* cultures only, although a slight bitter flavour was often present in the control cheese. The body and texture of the inoculated cheese were frequently mellower and more mature than those of the control cheese.

Although the present investigation was somewhat exploratory, it appears that further study on the addition of proteolytic strains to cheese is warranted. It would also be advantageous to study more extensively the effect of *L. casei* and *Sarcina lutea* strains on cheese flavour development since these strains in particular indicated flavour improvement by their use in this series of experiments.

It should also be pointed out that leucine cannot be considered the sole flavour-producing constituent of cheese, but, at the time of this investigation, it appeared to be the most suitable amino acid to use as a criterion for culture selection.

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TOXICITIES OF CERTAIN INSECTICIDES TO THE SWEETCLOVER WEEVIL, *SITONA CYLINDRICOLLIS* FAHR. (COLEOPTERA: CURCULIONIDAE), AND THE PROTECTION OF SEEDLING CROPS[1]

W. R. Allen[2] and W. L. Askew[3]

Field Crop Insect Section, Entomology Laboratory, Brandon, Manitoba

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ABSTRACT

In the laboratory, dieldrin, heptachlor, and parathion were similar in toxicity to the sweetclover weevil, DDT and malathion were less toxic, and toxaphene was the least toxic insecticide used. Under field conditions, dieldrin and heptachlor at half-a-pound per acre gave greater protection to sweet clover seedlings than parathion, DDT, or Metacide, and increased both the number of plants surviving and the dry weight per plant at harvest.

INTRODUCTION

Observations in Manitoba in 1953 confirmed the widely held suspicion that the sweetclover weevil, *Sitona cylindricollis* Fahr., may be inordinately destructive to sweet clover crops as the seedlings merge. Serious crop damage was observed in early May even during a period of cool wet weather.

The effectiveness of several insecticides for the control of this weevil has been reported (2, 8). Reduction of injury to emerging seedlings of sweet clover (4) and increases in stand and vigour of sweet clover crops have been shown (8). This is a report on the toxicities of several of these insecticides to the sweetclover weevil and the effectiveness of certain of them in protecting seedling crops.

METHODS AND MATERIALS

Laboratory Tests

Sweetclover weevils collected from the same field from September 15 to October 28, 1953, were used to establish dosage-mortality lines for each of the following insecticides:

Parathion: *0,0*-diethyl *0-p*-nitrophenyl phosphorothioate, redistilled and supplied by H. Hurtig, Entomology Section, Defence Research Board, Suffield Experimental Station, Ralston, Alta.

Dieldrin: 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a-5,6,7,8,8a-octahydro-1,4,5,8,-dimethanonaphthalene, recrystallized, 99.5 per cent pure (Shell Chemical Corporation, Agricultural Chemical Division, Denver, Colo.).

Heptachlor: 1 (or 3a), 4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene, 99.89 per cent pure, melting point 93°-95° C. (Velsicol Corporation, Chicago, Ill.).

DDT: 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane, melting point 108°-109° C., recrystallized by W. W. Hopewell, Entomology Section, Defence Research Board, Suffield Experimental Station, Ralston, Alta.

Malathion: 0,0-dimethyl phosphorodithioate, S ester with diethyl mercaptosuccinate, 96 per cent pure (American Cyanamid Co., New York, N.Y.).

Toxaphene: Chlorinated camphene, chlorine content 67-69 per cent (Hercules Powder Co., Wilmington, Del.).

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[1] Contribution No. 3300, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

[2] Associate Entomologist.

[3] Assistant Technician.

The weevils were sprayed in a spray tower fitted with a nozzle of the type designed by Hewlett (5). The insects were exposed in crystallizing dishes (diameter 12.5 cm.) placed at three locations within the sprayed area. The average spray deposit at each location, calculated for one dosage level using the dye dilution technique (7), was 1.145 ± 0.022 μgm. per sq. cm., with a confidence interval of 95 per cent. Twenty test insects anaesthetized with carbon dioxide were placed on heavy crepe filter paper (Reeve Angel No. 230) in crystallizing dishes; 4.0 ml. of spray were delivered and allowed to settle two minutes.

The sprays were applied as emulsions containing the appropriate amount of insecticide, 4 per cent by volume solvent (2 volumes Deobase oil[1] and 1 volume Cyclohexanone[2]), and 0.003 per cent by volume Triton X100[3]. The emulsion ingredients exclusive of insecticide were non-toxic to the weevils at the concentrations used.

After spraying, the weevils were held in 2-quart sealers for 3 days at room temperature ($21 \pm 3°$ C.), relative humidity 64 ± 10 per cent, until mortality counts were made. Fresh food was added at 12 and 48 hours. The percentage mortality was calculated by totalling the number of dead and moribund weevils. Those showing no movement when individually examined were considered dead; those capable of movement but unable to stand were considered moribund and invariably died within 3 days. Percentage mortalities were corrected by Abbott's formula (1); the control lots of weevils were sprayed with insecticide-free emulsion. The dosage-mortality lines were established on a total of 240 weevils at each of 4- or 5-dosage levels; two tests were completed on different days, each consisting of duplicate determinations on three groups of 20 weevils. The results were analysed by probit analysis (3). The confidence limits of the LD_{50}'s were calculated by the method of Litchfield and Wilcoxon (6).

Field Tests

In 1953 at Morden, Manitoba, a 1.4-acre block of sweet clover was seeded on May 30 at a rate of 20 lb. of seed per acre. On June 8, when half the seedlings had emerged, the seeded area was divided into seven plots; six plots were treated, and one was left untreated.

The insecticides tested were emulsion concentrates of:

Metacide, 30.7 per cent: (Pittsburgh Agricultural Chemical Co., New York, N.Y.).
Heptachlor, 25 per cent: (Velsicol Corp., Chicago, Ill.)
Dieldrin, 18½ per cent: (Shell Chemical Corp., Agricultural Chemical Division, Denver, Colo.).
Parathion, 25 per cent: (Thiophos, North American Cyanamid Ltd., Toronto, Ont.).
DDT, 25 per cent: (Canadian Industries Ltd., Montreal, Que.).

[1] Thompson Hayward Chemical Co., Kansas City, Mo.
[2] Eastman Kodak Co., Rochester, N.Y.
[3] Rohm and Haas Co., Philadelphia, Pa.

The sprays were applied with a low-pressure low-volume weed sprayer as follows: DDT at 1.5 lb. (of toxicant) per acre; Metacide at 1.0 and 0.5 lb.; parathion, dieldrin, and heptachlor at 0.5 lb.

The initial mortalities given by the spray deposits were determined by caging weevils on the plots 8 hours after spray application. The cages covered an area 1 square-foot and consisted of a cylindrical iron frame (2- and 3-inch wide galvanized material) covered with plastic screen. Five cages were placed on each plot and 50 weevils per cage were added. The percentage mortalities, corrected by Abbott's formula (1), were determined four days after treatment.

The percentage changes in the numbers of seedlings established on 5 one-yard lengths of drill row, selected at random, were determined by making plant counts on June 8 and 29, and July 16. The amount of defoliation during the same intervals was measured by counting the number of seedlings that had lost half of their total foliage because of weevil feeding.

To compare the stands of sweet clover established on the plots, the number and dry weight of plants (74.5 per cent of fresh weight) were determined on July 27 on 10 one-yard lengths of drill row selected at random from each quadrant of the plots. Significant differences were not found between the quadrants of each plot.

RESULTS AND DISCUSSION

Laboratory Tests

Table 1 shows that parathion, heptachlor, and dieldrin were similar in toxicity to the sweetclover weevil; that DDT and malathion were somewhat less toxic; and toxaphene was least toxic. Also, as the deviation from parallelism was not significant (6) for parathion, dieldrin, malathion, or toxaphene, the LD_{50} values may be used to express the relative potencies of these insecticides.

TABLE 1.—TOXICITIES OF VARIOUS INSECTICIDES TO *Sitona cylindricollis* FAHR. ON BASIS OF MORTALITY 3 DAYS AFTER SPRAY APPLICATION

Insecticide	Regression equation	LD_{50} μgm. per sq. cm.	LD_{50} 95 per cent confidence limits
Parathion	$Y = 5.48x - 0.24$	0.90	0.75 to 1.08*
Heptachlor	$Y = 3.55x + 1.22$	1.15	1.06 to 1.24
Dieldrin	$Y = 5.39x - 0.92$	1.26	1.19 to 1.34
DDT	$Y = 2.30x + 4.54$	1.59	1.42 to 1.78
Malathion	$Y = 5.85x + 3.64$	1.71	1.35 to 2.17*
Toxaphene	$Y = 5.01x + 1.07$	6.08	5.74 to 6.44

* Adjusted for heterogeneity.

TABLE 2.—PERCENTAGE CHANGES IN THE NUMBER OF SWEET CLOVER SEEDLINGS AND PERCENTAGES OF SEEDLINGS HALF-DEFOLIATED IN 5 ONE-YARD LENGTHS OF DRILL ROW FROM EACH PLOT; AND AVERAGE NUMBERS OF LIVE WEEVILS PER SQUARE FOOT (5 SAMPLES). COUNTS MADE JUNE 29 AND JULY 16, 3 AND 5 WEEKS AFTER APPLICATION OF INSECTICIDES, AT MORDEN, MANITOBA, 1953

Pounds of insecticide per acre		Three weeks after spraying			Five weeks after spraying		
		Percentage change in number of seedlings	Percentage of seedlings half defoliated	Av. number live weevils per sq. ft.	Percentage change in number of seedlings	Percentage of seedlings half defoliated	Av. number live weevils per sq. ft.
Metacide	1.0	+ 5.2	57	3.4	+15.0	26	5.0
Heptachlor	0.5	+ 2.1	46	4.2	+ 7.0	20	2.4
Dieldrin	9.5	0.0	51	0.6	0.0	0	2.0
Parathion	0.5	− 6.6	95	3.4	− 1.0	15	2.4
DDT	1.5	− 9.2	89	3.6	− 8.2	61	3.0
Metacide	0.5	−17.0	100	3.8	− 5.0	90	2.2
Control	0.0	−18.0	100	3.8	−10.0	80	1.4

Field Observations and Tests

At Morden, Manitoba, in 1953 the spring damage to seedling sweet clover by overwintering weevils was closely observed. A crop (1.4 acres) was sown on April 29 within 50 yards of a 1952 seedling crop (15 acres) that had been completely destroyed in August by a population of weevils approximating 400 per square foot. An excellent stand of clover was established May 8, but weevils accomplished a surprising amount of damage during ten days of cool wet weather that followed. On May 18, 45 per cent of the seedling crop was completely destroyed and the population was estimated at 19 weevils per square foot; and the remainder of the crop was 50 per cent thinned by a population of 3 per square foot. From May 11 to 18 the average daily maximum, minimum, and mean temperatures were 55°, 34°, and 44° F.; rainfall amounted to 1.12 inches, including 0.14 inch recorded as snow on May 11. These observations show the importance of applying insecticides early for adequate crop protection.

Table 2 shows that three weeks after spray application the number of seedlings had decreased in the parathion, DDT, and Metacide (0.5 lb.) plots and in the control, whereas the number of seedlings was maintained or increased in the Metacide (1.0 lb.), heptachlor, and dieldrin plots. Also, the foliage damage to the seedlings was less for the treatments that gave protection from seedling destruction. Five weeks after spray application the number of plants increased on all but the dieldrin and DDT plots, indicating that in general the plants continued to emerge during the fourth and fifth weeks. The decrease in defoliation suggests, also, that the plants were outgrowing early damage.

The initial mortality of weevils entering the plots after the sprays were applied on June 8 was probably high, for weevils were caged on the plots eight hours after spraying and four days later the percentage mortalities were: Metacide (1.0 lb.), 99; heptachlor, 93; dieldrin, 100; parathion,

TABLE 3.—AVERAGE NUMBERS AND DRY WEIGHTS OF SWEET CLOVER PLANTS JULY 27 IN PLOTS SPRAYED WITH VARIOUS INSECTICIDES JUNE 8, 1953. SAMPLE, 10 ONE-YARD LENGTHS OF DRILL ROW

Insecticide	Pounds per acre	Number of plants	Total dry weight, gm.	Dry weight/plant, gm.
Dieldrin	0.5	441*	41.8**	0.095***
Heptachlor	0.5	388	31.2	0.080
DDT	1.5	361	22.0	0.061
Parathion	0.5	360	23.2	0.064
Metacide	1.0	329	23.0	0.070
	0.5	277	11.8	0.044
Control	—	276	7.1	0.026

* Difference necessary for significance at 5 per cent level, 89; at 1 per cent level, 122.
** Difference necessary for significance at 5 per cent level, 6.4; at 1 per cent level, 8.7.
*** Difference necessary for significance at 5 per cent level, 0.008; at 1 per cent level, 0.011.

98; DDT, 91, and Metacide (0.5), 98. Table 2 shows that only plots treated with dieldrin showed a low population three weeks after spraying and indicates that the rest of the plots were subjected to equal populations during the 5-week period.

Table 3 shows that the number of plants established was greater in the dieldrin and heptachlor plots than in the check. There was a significant increase in yield of plants on a dry weight basis for all treatments except Metacide at 0.5 lb., and dieldrin gave a significantly better yield than heptachlor. It is of particular interest that insecticide treatments produced a significant increase in the dry weight per plant, especially dieldrin and heptachlor.

The results suggest that seedling stands of sweet clover are improved by insecticides that have persistent residues, because young seedlings protected from destruction and defoliation have the advantage of a longer period of growth to produce heavier plants.

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MAGNESIUM DEFICIENCY IN APPLE IN BRITISH COLUMBIA[1]

C. G. WOODBRIDGE[2]

Canada Department of Agriculture, Summerland, B.C.

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Abstract

Magnesium deficiency (leaf blotch) in apple in the interior fruit-growing areas of British Columbia was found to be widespread. In some orchards there was only a slight deficiency, few leaves showed symptoms of leaf blotch, and the vigour and productiveness of the trees did not appear to be affected. In others, the deficiency was marked, severe defoliation occurred, and few apples sized and matured. Soil applications of magnesium salts were not effective in preventing symptoms of the deficiency, but foliage spray applications, using 2 per cent solutions of magnesium sulphate, were effective. When the deficiency was severe, as many as eight sprays were required to correct it; and when the deficiency was slight, one spray was beneficial. When the magnesium content of leaves fell below 0.18 per cent magnesium on a dry weight basis, leaf blotch symptoms were invariably present and when the content was above 0.26 per cent, no symptoms were observed. Jubilee and Newtown varieties were more susceptible to leaf blotch than were other varieties grown in this area.

For many years a "leaf blotch" condition has been observed in apple orchards in widely scattered areas of the interior fruit-growing sections of British Columbia. In some seasons the trouble is slight and is of little or no economic importance. In others, the trouble is much more widespread and in a few orchards the disorder may cause severe defoliation. On susceptible varieties as much as 90 per cent of the leaves may fall by the end of September, leaving fruit small and unmarketable. The results of experiments have shown that this trouble is due to a magnesium deficiency in the tree tissues. It is the purpose of this paper to discuss this problem, to describe the details of some greenhouse and field experiments, and to present the results of chemical analyses on leaf samples collected in connection with this study.

Workers in several countries (1, 2, 4, 7, 8) have described symptoms of leaf blotch as found on the leaves of many different varieties of apple trees growing under different horticultural conditions. The literature is extensive and no attempt will be made to review it here in detail. It should, however, be noted that Wallace (8) in 1925 first described the symptoms of magnesium deficiency on apple being grown in sand culture. More recent work by Wallace (9) and by Hill and Johnston (4) described the symptoms of magnesium deficiency in orchards. These workers intimated that the symptoms of leaf blotch in the field and those on the leaves of trees growing in a magnesium deficient culture in a greenhouse were similar. They also noted that there was a correlation between the presence or absence of symptoms and the magnesium content of leaves.

Several workers (3, 4, 5) have reported that they experienced difficulty in obtaining satisfactory control of leaf blotch in the field. Soil applications of magnesium salts have sometimes been of no avail (5), but at other times they have given a slow or partial response (3). Mulder (7) reported that

[1] Contribution No. 277 from the Chemistry Division, Science Service.
[2] *Present address:* Department of Horticulture, State College of Washington, Pullman, Wash.

FIGURE 1. Symptoms of magnesium deficiency on apple leaves.
(a) Above—McIntosh. *(b) Below*—Newtown.

FIGURE 2. Severe magnesium deficiency on Newtown. Some leaves have fallen and many of those remaining show varying amounts of scorch. The fruit is small and immature.

in some of his experiments, four sprays of 2 per cent magnesium sulphate were applied at approximately 2-week intervals and that, whereas control of the symptoms was not quite complete, there was a marked improvement of the sprayed trees.

SYMPTOMS

Symptoms associated with magnesium deficiency vary considerably from variety to variety, orchard to orchard, and year to year. They are first distinguishable as brown necrotic or scorched areas between the veins of the affected leaves (Figure 1*a*). On some varieties, notably Newtown, the interveinal blotch is not prominent but the margin of the leaf may become chlorotic. This yellow colouration appears as a band which is broadest at the tip of the leaf and tapers off toward the basal end (Figure 1*b*). On others, the portions of the leaf lying adjacent to the main vein may become bright yellow. Sometimes the interveinal lesions coalesce and cause the leaf to shrivel, curl, and drop prematurely (Figure 2). With severe magnesium deficiencies, 80 to 90 per cent of the leaves of the affected portion may drop during the earlier part of the fall, so that the crop of fruit is unsized, immature, and a complete economic loss. Frequently only a single branch or a portion of a tree will exhibit the symptoms, but at times the whole tree may be affected. Such a tree is seldom entirely free of the disorder unless remedial measures are taken. Under orchard conditions, until a tree becomes of bearing age or on mature trees with a light crop, symptoms of the deficiency are not marked. On trees with a heavy crop they are more pronounced. When the deficiency is marked the tree or portion of the tree that is affected is in poor vigour and growth is retarded. When the deficiency is slight there seems to be little or no effect on vigour and growth. Other than being small in size there are no visual symptoms of magnesium deficiency in apple fruit. The symptoms appear earlier and are frequently more severe in wet years than in dry years. In the former they may appear late in July, whereas in the latter they do not show up until late in August and more frequently not until some time in September.

In sand culture, symptoms may appear within two years if the nutrient solution contains very low amounts of magnesium. Under this condition some of the lower buds on the previous year's growth fail to break dormancy and die (Figure 3).

Under the conditions prevailing in the Okanagan Valley, Jubilee and Newtown apple trees are most prone to symptoms of magnesium deficiency; McIntosh, Jonathan, and Rome Beauty are less susceptible; while Delicious and Winesap varieties seldom show leaf blotch.

EXPERIMENTAL PROCEDURES AND RESULTS

Sand Culture Experiment

In February 1949, 20 McIntosh (Rogers, N.Y.) apple trees on East Malling clonal root stock II were planted in washed sand in 3-gallon, asphalt-coated, galvanized containers. The trees were sub-irrigated with a nutrient solution using a semi-automatic arrangement as described by

Lott (6), but with a slight modification in that the ground glass return and control valve was replaced with a Bunsen valve. The normal nutrient solution used had a composition as suggested by Hoagland and the others varied only with respect to their potassium and magnesium content. There were five variations or treatments:

Treatment 1	2 × normal K		normal Mg
Treatment 2	normal K		normal Mg
Treatment 3	normal K	1/4	normal Mg
Treatment 4	normal K	1/16	normal Mg
Treatment 5	2 × normal K	1/64	normal Mg .

There were four trees in each treatment. At the start of the experiment the solutions were renewed every second week but as the trees grew and transpiration increased they were changed weekly.

In June of the first year of the experiment it was necessary to prune the trees because growth had been so great. At that time leaf and twig samples were collected, dried in an oven at 78° C., ground to pass a 20-mesh screen, and stored in air-tight containers for analysis. As there were no symptoms of magnesium deficiency, the concentrations of this element in Treatments 4 and 5 were changed to 1/64 and nil respectively. At the end of the growing season, the containers holding the trees were set in a shallow pit outdoors and wood shavings were placed about them to prevent winter injury. Samples of leaves and twigs were collected and treated as above. There were no leaf blotch symptoms.

In February of the second year (1950), the containers and trees were returned to the greenhouse. The sand in the containers was flushed with tap-water and rinsed with distilled water. No magnesium was added to Treatments 4 and 5. During the last week of May the trees were again pruned and samples of leaves and twigs collected. There were no symptoms of magnesium deficiency.

In October, when the trees were placed outside in shavings for the winter, it was noted that many leaves on the trees growing in nutrient solutions containing no magnesium were somewhat pale in colour and a few had some interveinal patches of necrotic tissue. These patches were more pronounced on leaves near the base of current growth than on those near the tip. Samples were collected for analyses.

In 1951 the trees were not brought into the greenhouse but were set up on a stand outdoors. The treatments were similar to those of the previous year but growth did not commence until April and then was much slower. Shortly after the trees commenced to grow, those in the no-added-magnesium solutions exhibited a marked lack of bud development. The leaves were small and pale. Typical leaf symptoms of magnesium deficiency appeared in August and they became much more pronounced in September. By the end of that month, many leaves had fallen (Figure 3) and the few fruits on these trees were smaller than those on trees receiving other treatments. The symptoms on the trees growing in Treatment 5 were more severe than those in Treatment 4.

The experiment was continued through 1952 and the symptoms of magnesium deficiency were more marked than those observed during the

FIGURE 3. *Left*—Defoliation of the lower leaves of McIntosh apple trees growing in a nutrient solution deficient in magnesium. *Right* Healthy McIntosh apple trees growing in a nutrient solution having an adequate supply of magnesium.

previous year. A comparison was made between the symptoms observed on the leaves of trees growing in the culture solutions containing no magnesium and leaf symptoms in several orchards. No difference was discernible.

Magnesium, potassium, and calcium were determined on the collected samples and a summary of the results is presented in Table 1. Determinations were made on aliquots of a solution of ashed plant material. Calcium was determined by the oxalate-permanganate method, magnesium indirectly by the colorimetric determination of phosphate after precipitation as magnesium ammonium phosphate, and potassium indirectly with nitroso-R salt after precipitation with sodium cobaltinitrite.

The data presented in Table 1 are representative of the results obtained when analyses were made on leaves collected at a given time. Similar cation interrelationships were noted in each set of samples. When the potassium: magnesium ratio in the nutrient solutions was changed from 2 : 1 to 1 : 1 (Treatments 1 and 2), the magnesium content in leaves increased from 0.16 per cent to 0.20 per cent on a dry weight basis; and when it was changed from 1 : 0 to 2 : 0 (Treatments 4 and 5), the magnesium content decreased from 0.10 to 0.08 per cent.

The variation of the calcium content of tissues with changes in potassium levels in the nutrient solutions follows that of magnesium. When the amounts in milliequivalents of the major cations present were totalled, it was found that the sums were almost equal (Table 1). This may be explained on the basis that the increased accumulation of one cation in leaves can only take place when the concentration of another is lowered. This reciprocal relationship may be due to a more or less constant requirement in the plant. Hence, if the potassium supply in the nutrient medium is sufficiently great to suppress the uptake of magnesium cations, the concentration of the latter may be reduced to such an extent that a magnesium deficiency may occur.

The relationships between the potassium, magnesium, and calcium content of twigs were found to be similar to those of leaves.

TABLE 1.—THE MINERAL CONTENT OF LEAVES OF McINTOSH TREES GROWING IN NUTRIENT SOLUTIONS. (AVERAGE FOR FOUR TREES. DRY WEIGHT BASIS)

Treatment No.	K: Mg	Magnesium	Potassium	Calcium	meq.*/100 gm.
		%	%	%	
1	2 : 1	0.16	2.83	1.01	135
2	1 : 1	0.20	2.38	1.11	132
3	1 : ½	0.12	3.39	0.97	145
4	1 : 0	0.10	2.51	1.23	134
5	2 : 0	0.08	3.04	0.93	131

* Milliequivalent.

Field Experiments and Observations

Two types of experiments were carried out in growers' orchards, viz., the application of magnesium compounds to the soil about affected trees, and the spraying of affected trees with solutions of magnesium sulphate.

(*a*) *Soil treatments:* Fall applications of magnesium salts (sulphate, chloride, and carbonate) were made in several orchards. In two orchards, a single treatment of magnesium sulphate was applied at the rate of 100 lb. per tree area—approximately 1600 sq. ft. In another two orchards, magnesium carbonate was applied at the rate of 25 and 50 lb. per tree area for three successive years. This experiment was duplicated using the sulphate instead of the carbonate. In a third set of two orchards, magnesium sulphate at the rate of 20 lb. per tree area was spread for four years around trees on which the leaves showed varying amounts of leaf blotch. Replicate treatments with magnesium chloride and carbonate were made. None of these treatments was effective in preventing the appearance of leaf blotch but in one orchard, after three years, there was an increase in the magnesium content of leaves (Table 2). Leaf samples were collected from each plot and were analysed for their mineral content. The results of these analyses are summarized in Table 3.

TABLE 2.—THE MAGNESIUM CONTENT OF LEAVES OF NEWTOWN APPLE TREES IN FIELD PLOTS TREATED WITH VARIOUS MAGNESIUM COMPOUNDS. (AVERAGES FOR FIVE TREES. PER CENT ON A DRY WEIGHT BASIS)

Treatment	Year sample collected	Magnesium compound applied		
		Sulphate	Chloride	Carbonate
——	1949	0.09	0.13	0.09
20 lb. (1949)	1950	0.15	0.14	0.12
20 lb. (1949-50)	1951	0.21	0.16	—
20 lb. (1949-50-51)	1952	0.21	0.21	0.19

TABLE 3.—THE MINERAL CONTENT OF MCINTOSH APPLE LEAVES FROM TREES IN PLOTS TREATED WITH VARIOUS MAGNESIUM COMPOUNDS. (DRY WEIGHT BASIS)

Treatment	Year sample collected	Magnesium	Potassium	Calcium
		%	%	%
$MgSO_4$ 50 lb. (1949-50-51)	1952	0.26	1.05	1.19
$MgCO_3$ 50 lb. (1949-50-51)	1952	0.18	1.07	1.29
No treatment	1952	0.23	1.00	1.24
No treatment	1952	0.24	1.37	1.38
$MgCO_3$ 25 lb. (1949-50)	1951	0.12	1.97	0.78
No treatment	1951	0.17	2.35	0.70

Soil samples were collected from each plot and the results of chemical examinations on these will be reported elsewhere. It should, however, be noted that exchangeable potassium was high and it is believed that this would tend to make difficult the control of magnesium deficiency by the use of soil additives. The soils varied from silts to coarse sands and their pH reactions ranged from pH 4.9 to pH 8.2.

(*b*) *Spray experiments:* Since earlier experimental work had demonstrated that dilute solutions of magnesium sulphate (2 lb. in 100 gal. of water) were of little value in lessening the symptoms of leaf blotch, more concentrated solutions (20 lb. in 100 gal.) were used in the tests now being reported. At all times when the dates of application of magnesium sulphate coincided with that for an insecticidal spray (D.D.T. and/or parathion), they were applied together. Trees selected for these experiments were moderately to severely affected with leaf blotch.

In a first test, 30-year-old Newtown trees were used and solutions of magnesium sulphate were applied eight times at one- to two-week intervals commencing in May. At times a readily discernible salt deposit was visible on the leaves but no adverse effects were noted. The leaves on the sprayed trees showed no signs of scorch. No defoliation was noticed in the latter part of the season, and the fruit sized well. Untreated check trees showed varying amounts of scorch and much defoliation towards the latter part of the growing season, and many of the fruits on these trees remained small and immature.

In a second trial, four sprays were applied to 25-year-old McIntosh and Newtown apple trees at 2-week intervals commencing in mid-June. There was no scorch on the McIntosh trees at the time the fruit was picked, but there was a small amount on the Newtowns. Examination of these trees a year after the sprays had been applied showed that there had not been an effective carry-over for scorch appeared on all trees.

To determine the most effective time of applying magnesium sulphate sprays, a 40-year-old McIntosh apple orchard was divided into seven plots adequately separated by check rows. Sprays were applied to every plot but the number and date of each application varied. The maximum number of sprays was seven, the minimum, one. It was concluded that the use of four sprays was most economical, that the sprays should be applied at about two-week intervals, that the first spray should be on by the middle of June, and that the final one should be applied by the end of July. The addition of magnesium to the calyx spray was not as effective as a later application and, as with a single early spray, only delayed the onset of symptoms. Once symptoms of leaf blotch appeared, the application of sprays did not lessen them but did prevent any further development.

In a final trial, magnesium sulphate (20 lb. in 100 gal. water) was applied with a concentrate type of spray machine to 20-year-old Newtown apple trees which were only moderately affected with leaf blotch. Two plots were used. Both received an application in June and one received a further application in July. At the time the fruit was harvested the plot receiving two sprays was free of leaf blotch symptoms. The amount of leaf blotch on the plot receiving only one spray was intermediate between that on the trees in the plot receiving two sprays and that on an untreated

TABLE 4.—THE MAGNESIUM CONTENT OF APPLE LEAVES. (PER CENT ON A DRY WEIGHT BASIS)

Variety	Remarks	Mg content
Newtown	Healthy, light crop	0.29
Newtown	Healthy, heavy crop	0.18
Newtown	Severe blotch	0.05
Newtown	Healthy, eight sprays	0.56
Newtown	Slight blotch	0.26
McIntosh	Healthy	0.26
McIntosh	Moderate blotch	0.12
McIntosh	Healthy, one spray	0.29
McIntosh	Healthy, six sprays	0.28
Delicious	Healthy, Orchard A	0.46
Jubilee	Healthy, Orchard A	0.26
Delicious	Healthy, Orchard B	0.24
Jubilee	Severe blotch, Orchard B	0.10
Newtown	Healthy, two sprays	0.38
Newtown	Slight blotch, one spray	0.24
Newtown	Moderate blotch	0.14

check. The magnesium content of leaves from some of these treated trees is listed in Table 4.

The results of chemical analyses on leaf samples from various orchards indicated that symptoms of magnesium deficiency may be present when the content of this element in apple leaves falls below 0.18 per cent on a dry weight basis. The lowest magnesium content determined was 0.05 per cent for Newtown leaves severely affected with leaf blotch. When the magnesium content was above 0.26 per cent deficiency symptoms were not observed. In Table 4 a few of the results obtained from analyses on Newtown and McIntosh are presented.

As a part of this study, a comparison was made between the magnesium content of leaves from Delicious, a variety that does not show marked symptoms of magnesium deficiency and some from Jubilee, a variety that shows marked deficiency symptoms. Samples were selected from two orchards in which Delicious and Jubilee trees were growing adjacent to each other. The results of analyses as presented in Table 4 show that the magnesium contents of samples from the first orchard were about double those in samples of the same variety from the second orchard. In the first orchard the magnesium content of *healthy* Delicious leaves was about double that of *healthy* Jubilee leaves. The same ratio held in the second orchard, but one sample was healthy and one diseased.

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BOTRYTIS LEAF SPOT ON ONIONS AND ITS CONTROL[1]

O. T. PAGE[2]

Ontario Agricultural College, Guelph, Ont.

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ABSTRACT

The small sclerotial neck-rot fungus, *Botrytis squamosa* Walker, was isolated from the margins of discrete, greyish-white depressed spots on the green leaves of bulbing onions (*Allium cepa* L.) grown on the Bradford Marsh, Ontario. In greenhouse pathogenicity trials there was no evidence of leaf spotting when seedling onions or maturing onions were inoculated with *B. squamosa* or with several other species of *Botrytis*. However, symptoms similar to those observed in the field, were induced on the green leaves of Yellow Globe onion plants when they were inoculated with *B. squamosa* and incubated in the laboratory under controlled conditions of light and temperature.

Significant increases in onion yields were obtained by the use of Manzate, Parzate (zineb), Vancide F-995W, or Orthocide 50W foliar sprays; DDT was included in all sprays to control thrips. These fungicides were demonstrated to be effective in controlling not only *Botrytis* leaf spot, but also downy mildew (*Peronospora destructor* (Berk.) Casp.).

INTRODUCTION

Forty-two hundred acres of bulb onions, with an annual value of approximately $2,500,000, have been grown in Ontario during each of the past three years. The commercial production of onions in the province is confined principally to areas of organic soil such as the Bradford, Leamington, Erieau, and Thedford Marshes. Onions grown in these areas are subject to several foliage diseases which, singly or together, have reduced yields substantially in seasons favourable for disease development. The most serious of these diseases in Ontario are downy mildew, *Peronospora destructor* (Berk.) Casp.; purple blotch, *Alternaria porri* (Ell.) Cif.; and leaf mould, *Stemphylium botryosum* Wallr.

In 1952, a leaf spot and wilt disease caused by *Botrytis squamosa* Walker was first recorded in Canada on the Bradford Marsh (8) where widespread injury to the foliage of yellow bulb onions occurred. During the same year, Viennot-Bourgin (10) observed, near Rennes, France, a similar spotting and wilting of the foliage of white Barletta onions caused by *B. squamosa*. Prior to these reports, Hickman and Ashworth (1) in England investigated a die-back disease of onion leaves, and concluded that *B. squamosa* was the predominant causal organism. In addition, Segall (9) in New York found that onion leaf spotting can be induced by the inoculation of onion leaves with several species of Botrytis including *B. allii* Munn, *B. cinerea* Pers., *B. tulipae* (Lib.) Lind., and *B. paeoniae* Oud.

In the present investigation, the causal relationship of *B. squamosa* with spot and wilt symptoms on onion leaves was studied. Field trials also were conducted to determine the effectiveness of foliar applications of certain fungicidal sprays in the control of *Botrytis* leaf spot and downy mildew.

[1] Contribution from the Department of Botany, Ontario Agriculture College, Guelph, Canada.

[2] Plant Pathologist.

DESCRIPTION OF BOTRYTIS LEAF SPOT

Onion leaves infected with *Botrytis squamosa* exhibited discrete, circular-to-elliptical, greyish-white, desiccated spots which during the early stages of infection had sharply delimited margins. The spots were slightly depressed, and varied from 1 to 10 mm. in length by 1 to 4 mm. in width; occasionally, contiguous spots fused. Characteristically, these necrotic spots were associated with a pendant withering of the leaf tips, particularly of the outer leaves of an onion plant. There was no evidence of sporulation on infected leaves examined in the field, although conidiophores were produced on infected leaves incubated in a moist chamber at room temperature. Microscopic examination of this material disclosed that the initials of conidiophores developed from vegetative hyphae in the host mesophyll and penetrated either through the stomata, or more commonly, directly through epidermal cells. The accordion-like folds in degenerated branches of mature conidiophores were similar to those illustrated by Walker (11), and are diagnostic of *B. squamosa*. The conidia are usually one-celled, and measured 12-22μ (mean 18μ) by 9-18μ (mean 13μ).

EXPERIMENTAL RESULTS

In a preliminary survey, four species of *Botrytis* were distinguished *in vitro* among 215 *Botrytis* isolates obtained from the leaves and bulbs of the common onion, *Allium cepa* L. *Botrytis squamosa* was the only species isolated consistently from necrotic spots on onion leaves whereas *B. squamosa*, *B. allii*, *B. byssoidea* Walker, and *Botrytis* of the *cinerea* type were isolated from diseased bulbs. Isolates of *B. squamosa* from the leaves of white, yellow, and red bulbing varieties, and from white onion bulbs, were morphologically similar when cultured in darkness on a semi-synthetic medium at a temperature of 25° C. Selected isolates of *B. squamosa* from onion leaves were used in pathogenicity tests on seedlings and maturing onion plants; several other species of *Botrytis* obtained from onion bulbs in the preliminary survey were compared with *B. squamosa* in some of the experiments.

Inoculation of Seedlings

It is probable that *B. squamosa* and also several other species of *Botrytis* overwinter in the soil in southern Ontario as sclerotia and possibly as conidia or mycelia. Inoculation experiments were performed to determine if seedling infection, particularly leaf spotting, could be induced by growing seedlings in infested soil. Data are presented in Table 1 for three soil-inoculation experiments conducted in a thermostatically-controlled, fan-vented greenhouse room maintained at a temperature of approximately 20° C.

Serious seedling rot did not result, in Experiment I, when Yellow Globe onion seeds were germinated beneath a half-inch layer of peat mulch infested with either *B. squamosa* or two other *Botrytis* fungi. However, when sclerotia or conidia of *B. squamosa* were applied directly to the testa of surface-sterilized seeds at the time of planting, 64 per cent and 20 per cent of the seedlings, respectively, were infected (Experiment II). In

TABLE 1.—INFECTION OF YELLOW GLOBE ONION SEEDLINGS INOCULATED WITH *Botrytis squamosa* AND INCUBATED IN A GREENHOUSE FOR THREE WEEKS AT A TEMPERATURE OF 20° C.

Experiment	Treatments		Seedlings	
	Method of application	Source of inoculum	Number examined	Percentage infected
I	Inoculum mixed with peat and applied as mulch over seeds	Mycelial suspension of *B. squamosa*	200	11
		Sclerotial suspension of *B. squamosa*	200	0
		Conidial suspension of *B. allii*	400	1
		Conidial suspension of *B. cinerea*	200	0
		Sterilized peat	200	0
II	Inoculum applied to tests of seeds	Sclerotia of *B. squamosa*	50	64
		Conidia of *B. squamosa*	50	20
		Sterilized peat	100	0
III	Inoculum atomized on soil and base of seedlings	Mycelial suspension of *B. squamosa*	225	77
		Conidial suspension of *B. allii*	150	80
		Conidial suspension of *B. cinerea*	300	72
		Sterile water	200	0

Experiment III, 77 per cent of the seedlings were infected by means of atomizing a mycelial suspension of *B. squamosa* on the soil and on the base of two-week-old seedlings; similar results also were obtained by applying aqueous inoculum of either *B. allii* or *B. cinerea*. A very humid environment was maintained throughout this experiment by covering each pot of seedlings with a polyethylene bag. It is probable that infection of seedlings by *B. squamosa* becomes serious only under extreme and abnormal conditions such as prevailed in Experiments II and III.

In a further experiment, the influence of soil temperature on seedling infection by *B. squamosa* and three other species of *Botrytis* was investigated. Onion seedlings approximately 7 inches high were transplanted into 5-inch glazed crocks, one plant per crock. Each crock contained either uninfested sand or sand infested with a mycelial mat of *B. squamosa*, *B. allii*, *B. byssoidae*, or *B. cinerea*. The mycelial mats were grown on liquid potato dextrose medium in 250 cc. flasks, and were washed with sterile water before blending with the sterilized sand. Six replications of each treatment were incubated for three weeks after transplanting in Wisconsin temperature tanks at 16°, 20°, 24°, 28°, or 32° C. The extent of tip wilt was evaluated by measurements made on 544 leaves of 120 plants.

Typical leaf spot symptoms were not induced, and no appreciable differences in the extent of tip wilt were observed among plants grown in sand infested with *B. squamosa* or the other *Botrytis* fungi. In general, the amount of tip wilt caused by the four species of *Botrytis* at 32° C. was more severe than at any of the lower temperatures investigated; regardless of temperature, negligible tip wilting occurred in control plants.

Inoculation of Maturing Onion Plants

Since no leaf spot symptoms were evident on more than 3,000 onion seedlings exposed to inoculum of *B. squamosa* and several other *Botrytis*

TABLE 2.—INFECTION OF MATURING YELLOW GLOBE ONION PLANTS INOCULATED WITH *Botrytis squamosa* OR OTHER *Botrytis* SPP. AND INCUBATED IN A GREENHOUSE AT A TEMPERATURE OF 20° C.[1]

Experiment	Treatments		Onion plants	
	Method of application	Source of inoculum	Number examined	Percentage infection[2]
I	Inoculum atomized on surface of leaves	Mycelial suspension of *B. squamosa*	45	1–24
		Conidial suspension of *B. allii*	30	1–24
		Conidial suspension of *B. cinerea*	30	0
		Sterile water	20	0
II	Inoculum injected into leaves	Mycelial suspension of *B. squamosa*	24	75–100
		Conidial suspension of *B. allii*	24	50–74
		Conidial suspension of *B. cinerea*	24	50–74
		Mycelial suspension of *B. byssoidea*	24	25–49
		Sterile water	24	0
III	Inoculum injected into centre of bulbs	Mycelial suspension of *B. squamosa*	41	1–24
		Conidial suspension of *B. allii*	12	1–24
		Sterile water	0	0

[1] Plants were incubated, following inoculation for 10, 16, and 20 days in Experiments I, II, III respectively.
[2] Expressed as percentage wilt of the leaf blades as measured from tip to sheath.

species, attention was directed to the inoculation of maturing onion plants. Because of the absence of suitable healthy control plants in the field, onion plants were grown and inoculated in a greenhouse room maintained at a temperature of approximately 20° C. The results of three inoculation experiments on maturing Yellow Globe onion plants are summarized in Table 2.

Yellowing and withering from the leaf tips downward, unaccompanied by spotting, resulted from atomizing the surface of onion leaves with an aqueous spore and mycelial suspension of either *Botrytis squamosa* or *B. allii* (Experiment I, Table 2). Droplets of water were maintained on the inoculated leaves throughout the above experiment by covering the plants with polyethylene bags and by atomizing the leaves daily. Extensive tip wilting was induced in Experiment II by injecting 1 cc. of *Botrytis* inoculum into the lacuna of an onion leaf by means of a hypodermic needle. Although this central hollow in an onion leaf blade provided an ideal environment, there was no evidence of leaf spotting. The possible systemic infection of onion leaf blades by *B. squamosa* and two other *Botrytis* fungi was investigated in Experiment III by injecting aqueous inocula of these fungi into the bulbous basal portion of maturing onion plants, proximate to their growing points. Again no leaf spotting occurred, but the leaves which emerged after inoculation were dwarfed, flattened, and curled. Control plants similarly injected with sterile water were unaffected.

Typical spotting of onion leaves as it was observed in the field was not produced in any of the foregoing, or in other unreported greenhouse trials conducted in 1951 and 1952. As a result, climatological observations made near experimental plots of onions at 8 a.m. and 5 p.m. daily were examined to determine if certain environmental conditions were associated

with the sudden and widespread appearance of *Botrytis* in these plots. The climatological data included the maximum and minimum temperatures, wet and dry bulb readings, a measurement of precipitation, and the amount of cloud for each of the two daily observation periods. These data, together with data on the influence of light on mycelial growth of *B. squamosa*, subsequently were assessed, and an experiment was designed to approximate the critical environmental conditions. Comparisons with other species of *Botrytis* were not made.

Maturing Yellow Globe onion plants were atomized with an aqueous suspension of *B. squamosa* isolate 190, and eight plants in duplicate trials were placed in each of the following selected environments:

(*a*) Sixteen hours of artificial light, temperature 25° C., alternated with 8 hours of darkness, temperature 12° C., for a total of 60 hours;

(*b*) Continuous darkness during 60 hours, temperature alternated as in (*a*);

(*c*) Inoculated plants maintained at either 12° or 25° C. in continuous darkness for 60 hours.

A high relative humidity was maintained by enclosing plants in polyethylene bags and by atomizing the foliage daily with sterile water. Uninoculated control plants were maintained in each environment.

In environment (*a*), discrete, circular, greyish-white depressed spots, with sharply delimited margins characteristic of field symptoms, were induced on the green leaves of Early Yellow Globe onion plants that had been atomized with a spore and mycelial suspension of *B. squamosa*. When sections of these leaves with spot lesions were placed on water agar in Petri dishes, the pathogen *B. squamosa* was re-isolated. Although a few atypical spots developed on several plants incubated in continuous darkness, the onion plants generally became chlorotic, particularly those maintained at a temperature of 25° C.

Fungicide Trials

The degree of control of *Botrytis* leaf spot and downy mildew obtained by spraying with some of the newer carbamate fungicides was investigated in spray trials conducted on the Bradford Marsh. Replicated blocks laid out in the same commercial field of Early Yellow Globe onions in 1952, 1953, and 1954, were divided into randomized plots each consisting of six 31-foot rows spaced 15 inches apart. Sprays at the rate of 100 gallons per acre were applied with a John Bean "Spartan" sprayer operated at a pressure of 150 pounds per square inch. This sprayer was modified to straddle the rows and to provide ample clearance about the crop. The boom, adjustable for row width and crop height, was equipped with six hollow-cone spray "Teejet" nozzles which were directed vertically downward over the rows; a quick shut-off valve permitted accurate metering of the spray solution.

Five spray treatments replicated five times were applied at regular intervals from July 10 to September 5, 1952. In 1953, six treatments replicated six times were applied from July 9 to August 21; eight treatments replicated five times were applied from July 14 to August 25, 1954. Onions,

TABLE 3.—TREATMENTS APPLIED AND YIELDS OF YELLOW BULB ONIONS FROM EXPERIMENTAL PLOTS, BRADFORD MARSH, 1952-54

Treatments[1]		Mean yield per acre[2]			3-year mean
Trade name	Pounds applied per acre	1952	1953	1954	
Parzate (zineb)	1.5	1326.8	1048.6	1129.6	1168.3
Manzate	2.0	1307.1	1057.5	1140.1	1168.2
Vancide F-995-W	2.5	—	1057.3	1006.0	—
Orthocide 50W	3.0	—	—	987.6	—
Nu-M	3.0	1159.8	863.4	896.8	973.3
Nu-Z	3.0	1137.5	940.2	924.5	1000.7
DDT	4.0	1199.3	932.6	850.8	994.2
	L.S.D. (5 per cent)	107.3	94.7	77.6	

DDT, a thripicide, and Triton B-1956, a depositing agent and sticker, were included in all treatments.
Mean yield of onions was calculated as the number of 50-lb. bags per acre.

from the middle 13.25 feet of each of the two centre rows of each plot, were harvested on September 12, 1952, on August 26, 1953, and on September 8, 1954, and yield data were recorded. The calculated yields per acre are presented in Table 3

The mean yields of onions in the fungicide-treated plots were each year significantly higher than in those treated with Nu-Z, Nu-M, or with DDT alone (Table 3). As the differences between the latter treatments were not significant, it appears that there was no zinc or manganese deficiency in the plots and that where Parzate or Manzate was used the increase in yield was due solely to their fungicidal action.

In 1952 and 1953 *Botrytis* leaf spot was severe in control plots by mid-August; in 1954 only a trace was present in the controls toward the end of the growing season. Downy mildew and leaf mould (*Stemphylium botryosum*) were severe in control plots in 1954, whereas these diseases appeared only sporadically in 1953, and were not observed in 1952. Carbamate sprays were effective in controlling not only *Botrytis* leaf spot (1952), but also mildew (1954).

DISCUSSION AND CONCLUSIONS

A spotting and wilting of onion leaves caused by *Botrytis squamosa* has been described herein; other investigators in England (1) and France (10) have observed a similar disease. A spotting of onion leaves caused by other species of *Botrytis* also has been reported in New York (9) where it has been referred to as "blast". It is to be noted, however, that the term "blast" was pre-empted by Jones (2) to describe a physiological spotting

and wilting of onion leaves. To separate two distinct disease conditions, "*Botrytis* leaf spot" is proposed as a descriptive and inclusive name for spot and wilt symptoms on the green leaves of onions caused by various *Botrytis* fungi, while the term "blast" would be retained for spot and wilt symptoms of a physiogenic nature as described by Jones.

Rot of seedling onions attributable to *Botrytis* fungi was not observed on the Bradford Marsh during three consecutive seasons. Apparently, the abundance of *Botrytis* inoculum, together with a favourable environment which resulted in seedling rot in the greenhouse, does not occur commonly in the field. McKeen (4) in reporting a severe rot of Spanish onion seedlings in Ontario caused by *B. allii* stated that, "it would appear that rather precise conditions must obtain before infection can become established". Attempts to demonstrate that leaf spotting might arise from the systemic infection of seedlings and of maturing onions by *Botrytis squamosa* and several other species of *Botrytis*, were unsuccessful.

Yellowing and softening, without spotting, resulted from attempts in the greenhouse to induce spot symptoms either by inoculating the surface of onion leaves with *Botrytis* inoculum or by injecting the inoculum into the leaf blades. These results are essentially in agreement with those reported by Walker (12) following inoculations of onion leaves with *B. allii*, *B. byssoidea*, and *B. squamosa*. Walker observed only softening of affected tissue, and concluded that there was little evidence that these three neck-rot fungi caused a destructive leaf blight. However, Munn (5) reported that leaf spot symptoms developed after atomizing a spore suspension of *B. allii* on onion leaves, providing the droplets of liquid were of sufficient size to prevent their drying off quickly. Viennot-Bourgin (10), readily induced spotting by inoculating the leaves of white onion with *B. squamosa*.

The present successful inception of disease symptoms on leaves inoculated with *B. squamosa* was accomplished only when the environment approximated that which occurred in the field under certain conditions. A diurnal alternation of light and darkness concurrent with an alternation of a relatively high temperature of 25° C. and a lower temperature of 12° C. was conducive to the development of typical leaf spot symptoms. Although the light period was essential for the retention of a vigorous green foliage on the onion plants, infection probably occurred during the dark period. A study of *B. squamosa* in culture has shown that mycelial growth is inhibited by a light intensity in excess of 100-ft. candles. The change from a higher to a lower temperature resulted in the formation of a condensate on the leaves, and thus provided the moisture necessary for infection to occur as predicated by Munn (5) and Segall (9). *Botrytis squamosa* when cultured on various media was found to make adequate mycelial growth at 12° C., although the optimum temperature for mycelial growth was approximately 20° C.

Significant increases in onion yields, obtained by the application of certain fungicidal sprays during three seasons, were the result of controlling, singly or together, *Botrytis* leaf spot and downy mildew; thrips were controlled by the addition of DDT in all sprays. Hitherto, the effective control of onion mildew with a carbamate-sulphur dust has been reported

by Nelson (6) in Michigan; mildew, "blast", and thrips were contolled by Newhall and Rawlins (7) with either Dithane D-14 spray or Dithane Z-78 dust, combined with DDT.

The effectiveness of fungicidal sprays was most evident in the present investigation during the three weeks prior to harvest. The retention of green foliage in fungicide-treated plots during this pre-harvest period permitted continued growth of the bulbs. The leaves of plants in plots sprayed with an insecticide alone collapsed and dried, curtailing bulb growth; similar behaviour was observed in adjacent unsprayed commercial fields. Delayed maturation of onions, attributed to the use of carbamate sprays in 1952, was not a factor in delaying harvesting in 1953 and 1954.

In 1952, McCall and Davis (3) reported increased yields of onions grown on organic soils in Michigan as the result of foliar applications of Nu-M or manganese sulphate; decreased yields resulted from foliar applications of Nu-Z or zinc sulphate. On the Bradford Marsh, differences in yields were not obtained from foliar applications of Nu-M and Nu-Z. Presumably deficiencies of manganese and zinc were not acute in the experimental plots, and yield increases, resulting from the application of carbamate sprays containing these minor elements, must be attributed to fungitoxic properties of the sprays.

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A COMPARISON OF PEROXIDE- AND OXYGEN-BOMB CALORIMETRY OF FEEDSTUFFS[1]

J. M. Bell[2]

University of Saskatchewan, Saskatoon, Sask.

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ABSTRACT

The caloric values of a series of feedstuff samples have been determined by the peroxide-bomb calorimeter and the oxygen-bomb calorimeter. The peroxide unit tended to overestimate the caloric values. The divergences between the two sets of values are attributed to the origin of certain correction factors and to the modern use of accelerators. The errors were found to display a negative correlation with the percentage of ash; and a regression equation for adjustment of results is presented.

INTRODUCTION

With increasing interest in accurate energy evaluations of feeds the use of calorimetry is increasing. It becomes of importance, therefore, to consider the relative merits of the two principal types of instruments now in use, viz., the oxygen-bomb calorimeter and the peroxide-bomb calorimeter. There is little, if any, difference in the skill required to operate the two instruments but the oxygen calorimeter is more costly to purchase although more economical to operate.

The most important difference between the uses of the two types of instruments lies in the correction factors required to obtain the true calorific values of the samples analysed. With the oxygen unit the procedure involves the ignition of a feed sample in the presence of oxygen under pressure and with a small amount of water. Corrections are then required in proportion to the amount of acid remaining in the bomb, the amount of fuse wire burned and the amount of sulphur in the sample. The relevant data are readily obtained by titration, measurement of unburned fuse wire and by sulphur determination.

In the case of peroxide calorimetry, the sample is mixed with twice its weight of accelerator ($KClO_4$) and thirty times its weight of sodium peroxide (Na_2O_2). With many types of feeds a further addition of 0.3000 gm. of benzoic acid/0.5000 gm. sample is required to facilitate proper ignition. Seven corrections must be made before the caloric value of the sample is obtained. Each one is discussed below.

Fuse Wire Correction

This accounts for heat generated by electrical resistance and oxidation of the wire itself. The same kind of wire is used for both types of calorimeters but the correction suggested for the peroxide unit is 0.005° F. for the fuse wire whereas for the oxygen unit the correction is 2.8 calories/cm. wire *burned*. While the latter is a more accurate procedure the difference probably would not be too serious in nutritional research.

[1] Presented before the Annual Meeting of the Associate Committee on Animal Nutrition, National Research Council (Prairie Regional Committee), Edmonton, Alta., February, 1955.

[2] Professor of Animal Husbandry.

Ash Correction

This correction concerns those chemical reactions between the inorganic matter of the sample and the alkaline fusion mixture which would not occur in ordinary combustion. The factor recommended (2) and based on fuel studies (4) is 0.005° F. deduction from the total temperature rise, for each one per cent of ash in the sample. The adoption of this correction factor in feedingstuffs analysis assumes that the ash in feed differs insignificantly in composition from that of coal or other feeds. No ash correction is required in the oxygen unit.

Sulphur Correction

This correction adjusts for the oxidation of sulphur from SO_2 to SO_3. It amounts to 0.010° F. for each one per cent of sulphur in the sample. Calculations suggest that the correction might be eliminated since most feeds contain less than 0.2 per cent sulphur, and since this omission would seldom result in overestimating the caloric value of a sample by more than 0.2 per cent of the true value.

Accelerator Correction

This deducts the heat generated by the decomposition of 1.0 gm. of $KClO_4$ and amounts to 0.2000° F.

Benzoic Acid Correction

This is similar to the accelerator correction but amounts to 2.313° F. for the 0.3000 gm. of benzoic acid ordinarily used.

Hydration Correction

This factor takes account of the heat of reaction of water with alkali. Factors have been derived for coals of various types and they vary from zero to 0.220° F./0.5 gm. sample; the higher values are obtained from the lignites or soft, low-carbon coals. If carbon can be taken as an index of hydration values the appropriate factors for feedstuffs may well exceed any reported for coals (Table 1). This correction is believed to be a very important one but there appears to be but little basis for the selection of a suitable value for nutritional research.

TABLE 1.—APPROXIMATE COMPOSITION OF CERTAIN CALORIGENIC MATERIALS

Sample	Ash	Carbon	Oxygen
	%	%	%
Peat	13.8	48.6	28.9
Lignite	11.0	64.7	17.4
Bituminous coal	7.5	81.0	4.8
Anthracite coal	7.9	87.0	2.0
Protein	Trace	50	Nil
Carbohydrate	Nil	40	50+
Fat	Nil	75	15

TABLE 2.—GROSS ENERGY VALUES OF FEEDS AND FECES AS OBTAINED WITH PEROXIDE- AND OXYGEN-BOMB CALORIMETERS

Sample	Ash (per cent)	Cal./gm. (oxygen)	Peroxide unit		Peroxide unit, adjusted[2]	
			Cal./gm.	Per cent error[1]	Cal./gm.	Per cent error
Starch	0.00	4.15	4.61	+11.1	4.20	+1.2
Wheat	2.17	4.50	4.89	+10.9	4.48	−0.4
Barley	2.22	4.50	4.86	+ 6.7	4.45	−1.0
Oats	3.70	4.59	5.08	+10.7	4.66	+1.5
Ration No. 6	4.61	4.38	4.83	+10.3	4.44	+1.4
Ration No. 1	5.30	4.44	4.71	+ 6.1	4.34	−2.3
Ration No. 2	5.55	3.88	4.24	+ 9.3	3.91	+0.7
Crested wheat grass	5.82	4.50	4.96	+10.2	4.57	+1.5
Linseed oilmeal	5.92	4.78	5.14	+ 7.5	4.74	−0.8
Feces, alfalfa, sheep	8.03	4.64	4.99	+ 7.5	4.62	−0.4
Alfalfa	8.74	4.42	4.83	+ 9.3	4.48	+1.4
Fish meal	12.35	5.26	5.36	+ 1.9	5.00	−4.9
Feces, brome straw, sheep	13.68	4.57	4.77	+ 4.4	4.47	−2.2
Feces, No. 4	15.17	4.26	4.58	+ 7.5	4.30	+0.9
Feces, No. 1	16.78	4.04	4.27	+ 5.7	4.02	−0.5
Feces, No. 12	19.78	3.88	4.24	+ 9.3	4.02	+3.6

[1] Percentage error as compared to the values obtained from the oxygen calorimeter.
[2] Adjusted by means of the regression equation.

'Water Equivalent' Correction

While the above corrections would appear to account for most of the subsidiary heat reactions, the water equivalent value of the instrument contains yet another adjustment based on the work of Parr (4) in 1900. It was demonstrated in his report that 27 per cent of the heat liberated by coal was due to the heat of combination of CO_2 and H_2O; therefore the heat resulting from the sample itself was only 73 per cent of the net heat produced after deducting the above corrections. This correction, based upon limited research and strictly on coal, results in the water equivalent of peroxide calorimeters (Parr type) being calculated as *true water equivalent* $\times$ 0.73 $\times$ 0.5 to allow for 73 per cent true combustion heat and for a 0.5 gm. sample.

This method of expressing the water equivalent probably should not be adopted for nutrition research without further clarification since (*a*) it was based upon a single type of coal; (*b*) it appears to duplicate, in part, several of the above mentioned corrections; (*c*) the use of accelerators has been adopted since Parr's work was published, and (*d*) the use of exactly 0.5 gm. of sample is meaningless in view of variations in composition. Nevertheless, the calculation of peroxide calorimeter results does require correction in addition to those mentioned above.

This report involves a comparison of the two types of calorimeters when used for feedstuffs analysis.

MATERIALS AND METHODS

Instrumentation

A Parr double-valve, plain-jacket oxygen bomb calorimeter and a Parr peroxide calorimeter were employed. Each had an auto-transformer and a Parr ignition unit in series to control the electric current. All operations were carried out in a thermostatically controlled room.

Samples

The feedstuffs analysed included commercial corn starch, cereal grains, cured roughages, high protein feeds of vegetable and animal origin, mixed rations, ruminant and non-ruminant feces. All samples were ground to pass through a 100-mesh screen and were analysed for ash content. They were dried at 105° C. prior to calorimetry.

Benzoic acid was added to all samples used in the peroxide unit.

Sulphur corrections were ignored in all cases.

RESULTS AND DISCUSSION

The results of the comparative analyses are shown in Table 2 in which the samples are arranged according to percentage of total ash. It is apparent that the peroxide unit produced caloric values that were consistently higher than those obtained from the oxygen type instrument. It is probable that factors other than proportion of ash to organic matter (or carbon) are involved in these discrepancies but a covariance analysis of percentage error with percentage ash revealed a significant correlation between the two. The correlation coefficient, r, was -0.485 whereas 0.482 was required for significance with $P = 0.5$.

As a result of this finding, a regression equation* was computed and the peroxide unit results were adjusted accordingly (*Table 2*). While it is true that this procedure has resulted in acceptable agreement of the values secured by the use of the two different instruments in most cases, it is likely that further research should be done, particularly on high-ash samples, before establishing a suitable correction factor for the peroxide calorimeter used in feed analysis.

CONCLUSIONS

A comparison of caloric values of various feedstuffs as measured by an oxygen bomb calorimeter and a peroxide bomb calorimeter, together with a study of the correction factors ordinarily used in connection with each instrument, suggests that appreciable errors may be incurred by uncritical application of the peroxide bomb calorimeter to such determinations. The following points are of particular interest:

1. The preponderance of calorigenic reagents over sample used in the peroxide unit increases the risk of extraneous heat reactions and of error, especially when sample composition is as highly variable as feeds are.

2. The correction factors currently in use were developed with heating fuels and prior to the modern use of accelerators to facilitate ignition and combustion, and are rather imperical in nature compared to those used in oxygen bomb calorimetry.

It has been demonstrated that ash content (and organic matter content) is correlated with errors observed and that a regression equation permits more accurate calculation of peroxide calorimeter data.

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* $Y = 8.025 - 0.2188X + 1.7753$, where X represents percentage of ash and Y represents percentage error compared to true value.

THE UTILIZATION OF LARD BY BABY PIGS[1]

H. M. Cunningham and G. J. Brisson

Canada Department of Agriculture, Ottawa

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ABSTRACT

Twelve 2-day-old baby pigs were fed liquid purified diets containing 1.5, 3.0, and 4.5 per cent fat. Seven-day digestibility trials were conducted at intervals until the pigs were slaughtered at nine weeks of age for carcass analysis.

The level of lard in the diet had no effect on the apparent digestibility of fat, casein or glucose, or on the efficiency of energy utilization. The fat digestibility increased from 83.8 to 90.3 per cent between the second and fourth week of one trial and from 91.3 to 97.6 per cent in another. There was no further increase from the fourth to ninth week.

The true digestibility of the lard was estimated from the fecal fat excretion of four additional pigs which were fed a fat-free diet from 2 to 56 days of age. The metabolic fecal fat excretion averaged 70.4 mg. per kilogram of body weight per day or 0.105 per cent of the dry matter intake. Level of fat in the diet affected the relationship between true and apparent digestibility of the fat. On the low, medium and high fat diets the true digestibility of the fat was 1.23, 0.70, and 0.59 per cent higher than the apparent digestibility.

INTRODUCTION

Recently there has been much interest in the formulation of sow's milk-replacement diets for baby pigs. Fat is an important ingredient in these diets and attention should be paid not only to the quantity in the diet but also to its fatty acid composition and digestibility.

The dry matter of sow's milk contains 30 to 40 per cent of fat (2, 8). Nelson (10) found that baby pigs would not survive without a fat supplement in their reconstituted skim-milk diet, but 10 per cent of fat appeared to be optimum in these and other trials (3, 4, 10). Lard was superior to butterfat for 2-day-old pigs (12), and this was attributed to its similarity to sow's milk fat.

Less satisfactory results were obtained with milk-replacement diets containing 20 to 30 per cent lard. Digestibility studies were not reported by any of the authors quoted above, and the possibility exists that lard might be insufficiently digestible to promote optimum growth. The digestibility of the lard might be affected by the concentration in the diet and high levels might affect the digestibility of other dietary constituents. A study was undertaken, therefore, of the extent to which pigs 1 to 8 weeks of age could digest lard, and the degree to which the digestibility was affected by the percentage fed.

[1] Contribution No. 281, Chemistry Division, Science Service.

TABLE 1.—COMPOSITION OF LIQUID DIETS

Ingredient	Gm. per kg. of "milk"		
	Diet 1 (low-fat)	Diet 2 (medium-fat)	Diet 3 (high-fat)
Crude casein	40.0	40.0	40.0
Lard[1]	14.0	28.0	42.0
Lecithin	1.0	2.0	3.0
Cerelose	65.0	50.0	35.0
Minerals[2]	9.9	9.9	9.9
Vitamins[3]	—	—	—

[1] The lard was homogenized with the lecithin before adding it to the milk.

[2] Same as those used previously (5).

[3] Vitamins in mg. per kg. of diet: thiamine, 1.28; riboflavin, 1.28; niacin, 4.26; choline chloride, 220.0; pyridoxine HCl, 1.28; folic acid, 0.052; para-aminobenzoic acid, 1.06; calcium pantothenate, 2.13; inositol, 10.64; biotin, 0.021; B_{12}, 0.064; alpha-tocopherol, 5.0.

Vitamin D_3, 400 I.U. per kg. of diet; vitamin A acetate, 1 drop containing approximately 28,000 I.U. was placed on the tongue of each pig weekly.

EXPERIMENTAL PROCEDURE

Yorkshire pigs were removed from their dams 48 hours after birth. They were placed in individual, wire-floored, metal pens equipped for the separate collection of urine and feces. The room temperature was maintained at 70° to 75° F. and during the first few days supplemental heat was provided with infra-red heat lamps.

Two similar experiments (Trials 1 and 2) were conducted. In each, six litter-mates were allotted in pairs of similar initial weight to each of three levels of fat intake. These levels were 11.5, 23.1, and 34.6 gm. fat per 100 gm. of dry matter, equivalent to 1.5, 3.0, and 4.5 gm. fat per 100 gm. of reconstituted 13 per cent solids "milk" (Table 1). The fat in each diet replaced an equal weight of cerelose. The "milk" was fed warm for the first 4 days and subsequently at refrigerator temperature. The pigs were weighed weekly.

Digestibility was determined between 1 and 8 weeks of age in four periods of two weeks each. Feed was provided ad libitum during the first five days of each period and then held constant at that level. During the last seven days of each period feces were collected four times daily, preserved with dilute HCl and toluene and stored in a refrigerator.* Upon completion of a collection period the feces were macerated in a Waring blender and weighed. Samples were drawn from each slurry and analysed for total lipids by the Saxon (6) method. Dry matter determinations were made on all samples from both trials while nitrogen and ash were determined on the feces from Trial 2 only.

* No collection was made during the third period of Trial 1 because several pigs had diarrhœa as a result of over-feeding.

The efficiency of the feed troughs in preventing feed wastage was checked by collecting the spilled "milk" with the urine and analysing for fat. Feed wastage was found to be less than one per cent in each of six determinations and hence was not considered in computing the digestion coefficients.

The fecal excretion of lipids from non-dietary origin (metabolic fecal fat) was determined in order to estimate the true digestibility of the lard. Four 2-day-old baby pigs were fed a diet similar to those given in Table 1 except that vitamin-free casein was used instead of crude casein, and cerelose was increased to replace the lard. The fecal collections began when the pigs were 10 days old and continued for three consecutive two-week periods.

The pigs in Trials 1 and 2 were slaughtered at the end of the ninth week. The heads, feet and viscera were discarded. The carcasses were split lengthwise along the mid line, and both halves were weighed. Five samples of the flesh and of the bones of one-half of each carcass were analysed individually for moisture, protein, fat and ash, and the composition of the whole carcass calculated.

RESULTS AND DISCUSSION

The coefficients of apparent digestibility of lard obtained in these trials are given in Table 2. An analysis of variance showed that the digestibility of lard was not affected by the percentage fed but was influenced by the age of the animals. Between the second and fourth week, the coefficients increased from 83.8 to 90.3 in Trial 1 and from 91.3 to 97.6 in Trial 2. There was no change thereafter. The coefficients obtained in Trial 2 were significantly higher ($P < 0.01$) than those obtained in Trial 1.

TABLE 2.—COEFFICIENTS OF APPARENT DIGESTIBILITY OF LARD

Weeks on trial	Low-fat diet		Medium-fat diet		High-fat diet		
	Trial 1						
	1[1]	2	3	4	5	6	Av.
1–2	89.3	84.0	88.2	74.9	88.1	78.5	83.8
3–4	93.7	88.2	91.4	87.5	91.7	89.2	90.3
8–9	92.7	88.0	93.9	95.0	94.7	92.2	92.8
	Trial 2						
	7	8	9	10	11[2]	12	Av.
1–2	92.3	88.0	92.8	90.3	93.7	90.5	91.3
3–4	99.2	96.9	97.0	97.0	97.9	97.4	97.6
6–7	91.6	93.8	97.5	97.9	99.9	98.6	96.6
8–9	95.5	90.0	97.6	98.8	100.0	97.3	96.6

[1] Pig number.
[2] Feces were voided only once or twice weekly from the 6th to 9th week and in negligible amounts.

TABLE 3.—SUMMARY OF COEFFICIENTS OF DIGESTIBILITY OF DRY MATTER

Weeks on trial	Low-fat diet	Medium-fat diet	High-fat diet
	Trial 1		
1–2	96.8	93.2	93.3
3–4	97.5	96.1	95.2
8–9	97.6	97.3	95.8
	Trial 2		
1–2	98.1	97.1	96.1
3–4	99.4	98.8	98.6
6–7	98.1	98.9	99.4
8–9	98.3	99.0	99.1

The four pigs receiving the fat-free diets weighed an average of 17.20 kilograms at 8 weeks of age. The fecal lipids excreted by these pigs averaged 70.4 mg. per kilogram of body weight per day ($r = 0.831$) or 0.105 per cent of the dry matter intake ($r = 0.780$). The value of 70.4 mg. per kilogram of body weight per day was employed in estimating the true digestibility. The average true digestibility of the low-, medium- and high-fat diets in Trials 1 and 2 were calculated to be 92.89, 93.54 and 94.09 per cent respectively. These figures are 1.23, 0.70 and 0.54 per cent higher than their respective average apparent digestion coefficients. Although the difference between the true and apparent digestibility of lard appears to be small, the metabolic fecal fat can account for a large proportion of the fecal lipids when the dietary fat is well digested. Thus the calculated metabolic fecal fat averaged 8.7 and 35.9 per cent of the total fecal lipids in Trials 1 and 2, respectively.

The apparent digestibility of protein was not affected by the level of lard in the diet but increased from 97.9 to 99.0 per cent between the age of 2 and 4 weeks. Terroine and Spindler (13) obtained similar apparent digestibility coefficients for cow's milk proteins with 8 to 10 kg. pigs. Glucose was digested to the extent of 99.6 per cent and was not influenced by either the age of the pigs or the level of lard in the diet.

The digestibility of dry matter (Table 3) increased with age as a result of the increase in digestibility of fat and protein. The data also indicate that there was a significant decline in the dry matter digestibility with increased fat intake. It has already been stated that the level of fat had no effect on the digestibility of protein, fat or carbohydrate and the fat in all diets was less completely digested than the casein and glucose. More fat was excreted by the pigs fed the higher levels of fat and this accounted for the greater output of fecal dry matter.

Data on body weight, feed efficiency, and carcass composition are presented in Table 4. The energy content of the diets was calculated by employing factors of 9.48 cal./gm. for lard, 5.86 for casein, and 3.76 for glucose (10). The greater 9-week total energy intake of the pigs fed the

TABLE 4.—AVERAGE WEIGHT GAIN, FEED EFFICIENCY AND CARCASS COMPOSITION OF PIGS IN TRIALS 1 AND 2

	Low-fat diet	Medium-fat diet	High-fat diet
Number of pigs	4	4	4
Initial mean weight (kg.)	1.382	1.457	1.375
Total 9-week "milk" intake (kg.)	146.04	144.26	118.25
Total 9-week energy intake (therms)	90.69	101.96	93.72
Daily gain (gm.)	200	227	204
Dry matter intake per gm. body gain (gm.)	1.56	1.34	1.23
Calorie intake per calorie in the carcass	4.27	3.20	3.54
Nitrogen intake per gm. of carcass nitrogen (gm.)	3.68	3.33	3.03
Protein in carcass dry matter (per cent)	49.7	40.4	43.3
Fat in carcass dry matter (per cent)	41.0	52.2	49.1
Ash in carcass dry matter (per cent)	9.3	7.4	7.6

medium-fat diet resulted in apparently faster growth rates and high average carcass fat content but the differences were not statistically significant. The dry matter intake per gram of body weight gain decreased significantly ($P < 0.01$) as the fat content of the diet increased but there was no difference in the feed efficiency when expressed as calorie intake per calorie in the carcass. This is in agreement with the work of Hoagland *et al.* (7) who compared the carcass composition of rats receiving levels of 5, 11 and 18 per cent fat. They found no statistically significant differences in weight gain or in the storage of fat, protein or energy.

The nitrogen intake per gram of carcass nitrogen decreased significantly ($P < 0.01$) as the level of lard was increased. This may be attributed to the fact that casein supplied a greater percentage of the energy of the low- than of the high-fat diets. The protein content of all diets exceeded the requirements of baby pigs (1, 11) and more was deaminated for energy by the pigs fed the low-fat diet.

As the lard content of the diet was increased, the incidence of diarrhœa increased, and there was a greater tendency for pigs to go 'off feed'. It was concluded, however, that the superiority of low-lard diets, observed by other workers, could not be attributed to differences in digestibility of the lard.

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PENETRATION OF AND PERSISTENCE IN SOIL OF THE HERBICIDE 3-(p-CHLOROPHENYL)-1,1-DIMETHYLUREA (CMU)[1]

L. A. Birk[2]

Chemistry Department, Ontario Agricultural College, Guleph, Ontario

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ABSTRACT

A study was made of the soil penetration and persistence of the herbicide 3-(p-chlorophenyl)-1,1-dimethylurea, applied in June, 1952, to a fallow loam soil at rates ranging from 1.6 to 64 lb. per acre. Residual concentrations at 0-2 in., 2-4 in., and 4-8 in. depths were determined by chemical analysis, and the cereals winter wheat and oats were used as indicator crops.

On most of the plots over 90 per cent of the CMU remained in the 0-2 in. layer during the season of 1952. While the summer was relatively hot and dry, there were several quite heavy rains. In the spring of 1953, following a period of heavy rainfall, chemical analysis indicated that on the majority of the plots an average of over 44 per cent of the CMU had penetrated below the 2 in. depth, 13.4 per cent now being in the 4-8 in. layer.

By the end of the first year approximately 90 per cent of the CMU had disappeared from all except the most heavily treated plots. During the second year the rate of loss was somewhat less, averaging about 62 per cent for all plots except the latter.

Based on the growth of cereal crops as indicators, CMU toxicity has persisted in the soil for relatively long periods. Three years after treatment, the 16 lb. per acre plots showed only 65 to 70 per cent survival of oats, and on the 32 lb. per acre plots only the odd oat plant survived.

INTRODUCTION

The herbicide 3-(p-chlorophenyl)-1,1-dimethylurea, commonly known as CMU (trade names "Karmex" W Herbicide and "Telvar" W Weed Killer) is of importance for weed control on croplands and for soil sterilization purposes. Owing to its low water solubility and relatively long-term herbicidal activity, questions concerning rate and extent of soil penetration and persistence in the soil are quite important. The majority of such studies have involved relatively low concentrations of CMU (e.g., 0.25 to 5 lb. per acre) (2, 4, 5) and/or the growth of indicator crops to determine the time required for the herbicide to drop below the critical concentration for plant growth (6, 7, 8). While the use of plant indicator methods is practical, it is nevertheless indirect and is desirably complemented by direct chemical analyses.

Frequently, the climatic conditions under which CMU studies were conducted have been very different to those prevailing in Ontario (8, 10). For instance, Sharp *et al.* (10) reported that at Manhattan, Kansas, corn and oats grew normally 10 months after the application of CMU at rates up to 160 lb. per acre; whereas Knowles (6) found at Ottawa, Ontario,

[1] Contribution of the Department of Chemistry, Ontario Agricultural College.

[2] Associate Professor.

TABLE 1.—RESULTS OF CHEMICAL ANALYSES OF SOIL SAMPLES AFTER TREATMENT WITH 3-(p-CHLOROPHENYL)-1,1-DIMETHYLUREA (CMU) ON JUNE 24/52*

Rate of application lb./acre	Soil layer (inches)	Amount found in lb./acre						
		June 25/52	July 8/52	July 29/52	Sept. 3/52	June 19/53	Aug. 31/53	June 21/54
1.6	0-2	0.73	0.39	0.11	0.12	0.16	0.04	0.08
	2-4		0.06	0.03	0.01	0.01		
	4-8		0.04	0.02	0.03	0.00		
8	0-2	6.71	2.33	1.48	0.21	0.41	0.00	0.24
	2-4		0.08	0.06	0.00	0.13	0.08	0.02
	4-8		0.16	0.02	0.04	0.10		0.02
16	0-2	15.84	5.50	3.79	0.53	0.97	0.08	0.36
	2-4		0.26	0.12	0.06	0.42	0.25	0.14
	4-8		0.47	0.12	0.15	0.21	0.31	0.10
32	0-2	35.13	11.45	9.01	2.63	1.06	0.13	0.48
	2-4		0.43	0.13	0.05	1.17	0.55	0.37
	4-8		0.98	0.22	0.12	0.45	0.34	0.16
64	0-2	69.04	28.12	22.55	14.72	5.15	0.37	1.83
	2-4		1.35	0.27	0.40	2.99	1.38	1.14
	4-8		1.43	0.44	0.25	0.73	0.81	0.65

* All rates and concentrations are expressed on an active ingredient basis. Certain of the lower soil layers were not analysed at the last two sampling dates, since it was felt that the amounts present would be insignificant. It should be noted that the 4-8 in. soil layer represents twice as great a volume as each of the other layers.

that, while corn and oats grew normally on soils which had received an application of 20 lb. of CMU per acre 15 months previously, they did not survive on soils which had been treated with 40 lb. per acre. Loustalot *et al.* (8) concluded that the persistence and effectiveness of CMU in the soil is dependent, to a large extent, on prevailing climatic conditions such as rainfall and temperature and upon certain other factors such as soil texture.

The present study was undertaken in order to obtain information on the rate and extent of soil penetration and the persistence of CMU, applied at rates ranging from 2 to 80 lb. per acre of the 80 per cent commercial product, in a soil of intermediate texture and under Ontario conditions. Residual concentrations have been determined by chemical analysis and the cereals fall wheat and oats have been used as indicator crops. Observations were also made on the control of couch grass, most of the plot area having been heavily infested with this weed previous to the beginning of the experiment.

MATERIALS AND METHODS

The experiment was conducted on the Soils and Agricultural Engineering Farm, Ontario Agricultural College. A fallow soil, classified as Burford loam, cultivated just previous to the CMU application, was used. On June 24, 1952, applications of 80 per cent commercial CMU were made on replicated plots one rod square at rates of 2, 10, 20, 40 and 80 lb. per acre (equivalent to 1.6, 8, 16, 32, and 64 lb. of active ingredient). The soil was sampled four times during the first season: 1 day, and 2, 5, and 10 weeks after the CMU application. While further sampling had been contemplated September proved to be a relatively dry month, so that it was felt that the soil penetration picture would not change appreciably. In 1953 the soil was sampled in June, approximately one year after the setting out of the experiment, and at the end of August. A final sampling was made in June, 1954, approximately two years after the commencement of the experiment. Sampling depths were 0-2 in., 2-4 in., and 4-8 in., from 24 to 30 cores being taken from each plot per sampling.

The acid hydrolysis micromethod of Lowen and Baker (9) for CMU, with a minor modification, was used for the chemical determination of residues in the soil samples. The procedure involves extraction of the CMU from the soil with a mixture of acetonitrile and glacial acetic acid, its acid hydrolysis to yield p-chloroaniline as one of the products, and a colorimetric determination of the latter. The modification of procedure introduced was a filtration through Whatman No. 42 filter paper previous to the colour development steps. The presence of undesirable suspended material at this stage was frequently encountered, and it was found that filtration resulted in clear solutions, with no loss of p-chloroaniline provided a suitable washing technique was used. Blank determinations were run on the soils of the control plots and results from the treated samples corrected accordingly.

Since the method of Lowen and Baker does not extract all the CMU, and the percentage extracted varies with the soil type, it is necessary to conduct recovery experiments (procedure given by Lowen and Baker) and apply a "Recovery Correction Factor" to all results. Furthermore, with the same soil the percentage recovery tends to be somewhat higher where heavier applications of CMU have been made than for lighter applications. It is customary to calculate and employ an average recovery factor, based on recoveries from a series of soils to which applications of CMU ranging from light to heavy have been made. For the soil of this study the average Recovery Correction Factor was found to be 1.65. We found that where the CMU content was low the use of this correction factor tended to give results about 10 per cent too low, and for soils of high CMU content about 10 per cent too high. All results recorded in Table 1 have been corrected by the use of this factor.

More recently Bleidner *et al.* (1) have developed another method of determining CMU residues in soils and plant tissue which gives somewhat higher (but not complete) recoveries. Although aware of this method early in 1953, it was considered inadvisable to change to another method in the midst of this study. It is felt that, in a comparative study such as this, even though the experimental data may not be as exact as one might wish, nevertheless, this does not detract from their usefulness from a practical standpoint.

Since rainfall and soil properties, such as texture and organic matter content, are quite important in determining the behaviour of CMU in a soil, precipitation data and the more pertinent soil data were obtained from the Soils Department, Ontario Agricultural College.

RESULTS AND DISCUSSION

The results of the chemical analyses of the soil samples are given in Table 1. In each case the recorded values represent the average of those of replicated plots. Agreement between the same horizons of similarly treated plots was, in general, very good, particularly during the first season. The variation between results for horizons in replicates increased after the first year, although total CMU contents checked quite well. Precipitation data are given in Table 2 and soil data in Table 3. The climatic and soil data should facilitate comparison of our CMU residue data with such data from other areas and should permit predictions as to the probable behaviour of CMU under conditions somewhat similar to those of this study.

In discussion of the Recovery Correction Factor it was pointed out that the percentage recovery was somewhat higher when heavier applications of CMU had been made than for lighter applications. The work of Sherburne and Freed (11) provides an explanation for this finding. These workers, in their studies on the adsorption of CMU in the soil, found that there is a statistically significant correlation between adsorption and the amount of clay in the soil. They also found a correlation coefficient of 0.991 for the relationship between the amount of CMU adsorbed by the soil and the organic matter content, They state that in soils high in organic

TABLE 2.—MONTHLY PRECIPITATION DURING EXPERIMENTAL PERIOD—INCHES OF RAINFALL

Month	1952	1953	1954
January to March* (inclusive)	—	4.99	9.46
April	2.85	2.49	4.98
May	3.17	6.38	1.39
June	1.30	4.14	2.48
July	3.26	2.40	0.62
August	2.48	2.63	4.50
September	2.73	2.52	2.98
October	1.06	0.88	7.87
November	2.74	1.97	2.65
December*	3.02	2.13	3.52

* Precipitation occurred as both snow and rain. All has been converted to, and is expressed as, rainfall. Data for this period and for the month of December are from the Physics Dept., O.A.C., Weather Station. All other weather data are from the Soils and Agricultural Engineering Farm.

TABLE 3.—CHEMICAL ANALYSES OF SOIL FROM THE PLOT AREA

Sample*	Mechanical analyses			pH	Organic matter	Max. water holding capacity	Moisture equivalent
	Sand	Silt	Clay				
	%	%	%		%	%	%
0-2 in.	55	24	21	7.0	3.70	55.83	23.80
2-4 in.	57	22	21	7.2	3.83	60.38	23.83
4-8 in.	56	24	20	7.2	3.83	61.01	23.98
0-8 in.	59	23	18	7.2	3.63	56.05	24.36

* All samples represent composites of the respective horizons.

matter the CMU treatment will be somewhat less effective. The work of Dallyn (3) corroborates this, since he found that a high concentration of organic matter in a soil reduced the effect of CMU. Sherburne and Freed further conclude that more immediate effects from CMU may be expected on sandy soils, but the chemical will be lost more readily than on heavier soils. When light applications of CMU are made a higher percentage of the applied CMU will be in the adsorbed state than will be the case when heavy applications are made. The adsorbed CMU will, of course, be more difficult to extract than that which is in soil solution.

Examination of Table 1 will indicate that in both 1952 and 1953, in the case of the late summer samplings the CMU values on the surface 0-2 in. layers were relatively low, frequently being lower than those obtained the following spring. This was more particularly true of those plots to which the lower applications of CMU had been made. In both seasons the late summer sampling times had been preceded by a period of very little rainfall, so that the surface soil was quite dried out. The lower than expected values can be explained on the basis of increased adsorption of the CMU by the clay colloids and organic matter, and hence increased difficulty of extraction; that is, as the surface soil dries out there will be less CMU in soil solution and more will have passed into the adsorbed state.

This is in accordance with the hypothesis advanced by Sherburne and Freed (11). Further, greater effectiveness of a given application of CMU from the standpoint of plant toxicity would be expected under fairly moist than under drier conditions. It appears that the late summer results for CMU are somewhat low and do not give a true picture of the actual CMU content of these soils. However, the results from the June and earlier season analyses would not be subject to the same error.

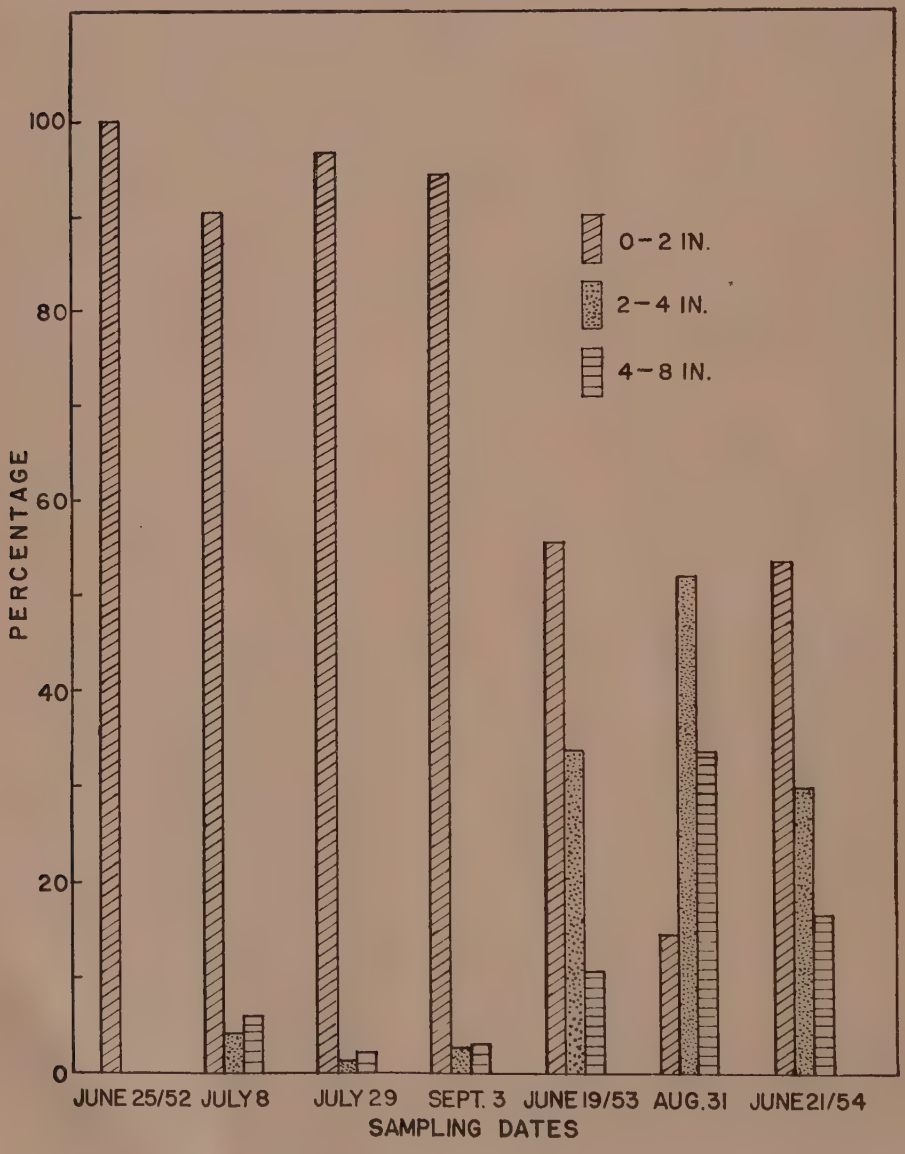

FIGURE 1. Over-all average distribution of 3-(p-chlorophenyl)-1,1-dimethylurea (CMU) in the different sampling layers.

Rate and Extent of Soil Penetration by CMU

In considering penetration data in Table 1 and Figure 1 it should be pointed out that, although the total rainfall during the 1952 sampling period was only 6.59 in., there were several quite heavy rains, one of 0.85 in. being recorded on July 18 and another of 1.00 in. on August 3. However, the season tended to be rather hot and dry. With the exception of the 1.6 lb. per acre plots, where apparently 77 per cent of the CMU remained in the 0.2 in. layer, from 89 to 94 per cent of the CMU did not penetrate beyond the 2 in. depth. From 2.4 to 4 per cent was present in the 2-4 in. layer, and from 3.1 to 6.7 per cent in the 4-8 in. layer. It should be noted that the 4-8 in. layer represents twice as great a soil volume as either of the other two layers, so that the concentration in p.p.m. of CMU in the 2-4 in. layer was actually greater than in the 4-8 in. layer.

In the spring of 1953, at the time of the June 19 sampling, in those plots which had received 8, 16, 32, and 64 lb. of CMU per acre, an average of 55.6 per cent of the CMU was present in the 0-2 in. layer, 31.0 per cent in the 2-4 in. layer and 13.4 per cent in the 4-8 in. layer. There was, however, considerable variation from treatment to treatment. The 1.6 lb. per acre plots showed a considerably higher percentage in the 0-2 in. layer, probably because of more complete adsorption of the smaller amounts of CMU which had been applied to them. The very appreciable downward movement of the CMU which had taken place by the time of the above sampling can be explained by the prolonged period of heavy rainfall which occurred in the spring of 1953. There was a total rainfall of 10.2 in. during the 8 weeks previous to the June 19 sampling, a rainfall of 2.19 in. being recorded on May 24. As indicated by Figure 1 the soil penetration picture was not changed appreciably one year later, on June 21, 1954.

Persistence of CMU in the Soil

The marked drop in the total CMU content of the treated soils during the season of 1952 is shown graphically in Figure 2. The drop was particularly precipitous during the first two weeks following the application of the CMU, and then became somewhat less so, continuing at a fairly uniform rate for the remainder of the summer; although as previously explained the results for the final sampling in 1952 (September 3) are very probably too low for the 0-2 in. layers, particularly for those plots which received the lower applications.

At the time of the second sampling, 2 weeks after application, the CMU contents ranged from 30.6 per cent of the original application, in the case of the 1.6 lb. per acre treatment, up to 48.3 per cent of the original application in the case of the heaviest treatment, with an over-all mean of 38 per cent. Hill *et al.* (5) have made a study of mechanisms relating to the disappearance of herbicides from soils. They point out that, since CMU is approximately 20,000 times less volatile than the isopropyl ester of 2,4-D, loss of CMU by volatilization from the soil is likely to be a minor factor under field conditions. This type of loss might have been suspected in this study, since the CMU was applied to the surface of fallow soil and the three days immediately following the application were sunny and

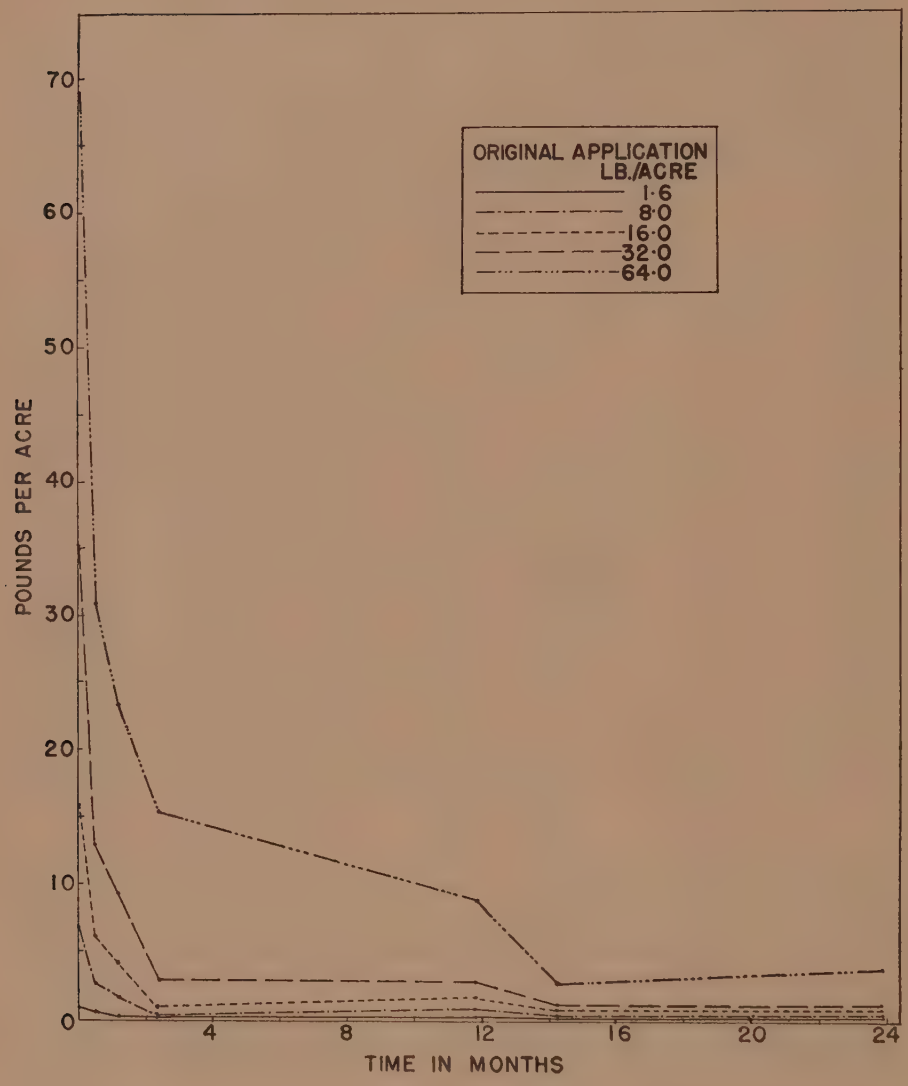

FIGURE 2. Chemical analyses of soils after treatment with 3-(p-chlorophenyl)-1,1-dimethylurea (CMU). Total amount found (0-8 in.).

without rain with an average daily maximum temperature of 93° F. A rainfall of 0.59 in. occurred on the fourth day, but even after this rain the more heavily treated plots still presented a whitish appearance due to the CMU. Following a total rainfall of 0.99 in. on July 3 and 4 this was no longer visible. Hill *et al.* also studied the role of photodecomposition in the fate of CMU and conclude from their work that, while this is likely to be a minor factor in more humid areas, it may be a positive factor in the disappearance of CMU from the soil when little rainfall occurs after application and the chemical remains on the soil surface. It appears quite

possible that photodecomposition was a factor in the disappearance of CMU during the first few weeks of the experiment. However, the above workers concluded that under field conditions the disappearance of CMU is due primarily to microbiological decomposition.

At the time of the June 19, 1953, sampling, approximately one year after the setting up of this experiment, the CMU contents of those plots which originally had received from 1.6 to 32 lb. of CMU per acre ranged from 8 per cent to 10.6 per cent of the original applications, the mean value being 9.2 per cent. The plots which originally had received 64 lb. per acre of CMU now contained an average of 13.8 per cent of this amount.

In June, 1954, two years after the beginning of the experiment, those plots which originally had received 8, 16, and 32 lb. per acre, respectively, now showed CMU contents ranging from 3.25 to 3.75 per cent of the original application with a mean content of 3.51 per cent of the original. The 64 lb. per acre plots now contained an average of 5.66 per cent of the original application.

The above data and the graphical presentation of Figure 2 indicate the rate of loss of CMU during the second year after application to have been much less than during the first year. Considering all treatments, except that of 64 lb. per acre, during the first year over 90 per cent of the CMU present originally disappeared, whereas during the second year only about 62 per cent of the CMU present at the beginning of that year disappeared. The rate of disappearance from the 64 lb. per acre plots was somewhat less each year, but also proportionally much less the second than the first year. Growth of indicator crops would suggest that CMU was lost at a somewhat similar rate during the third as during the second year.

Lateral Movement of CMU in the Soil

Owing to its low water solubility and slow rate of soil penetration, CMU applied on sloping soil is subject to washing along with the surface soil on to adjacent untreated areas. While this undesirable characteristic of CMU was not realized at the time this experiment was begun, fortunately the area chosen was quite level; so that washing or lateral movement of the applied CMU has played a minor role in this experiment. However, in the case of a few of the plots there has been some evidence of washing. To test the extent of washing a strip 3 feet wide alongside one series of 40 and 80 lb. per acre plots, where there was a slight slope away from the plots, was sampled in June, 1954, at the 0-2 in. depth. CMU was found to be present in the surface 2 inches of soil to the extent of 0.20 lb. per acre.

Growth of Indicator Crops

Beginning with the fall of 1952 the cereal crops winter wheat (Cornell 595) and oats (Beaver) were sown each fall and spring, respectively, along one side of one plot from each treatment. The rest of these plots was left undisturbed to permit further soil sampling. The survival of winter wheat was estimated in the spring. In calculating survival data only reasonably thrifty-appearing plants were counted. In the spring of 1955, three years

TABLE 4.—SURVIVAL OF WHEAT AND OATS ON CMU TREATED PLOTS

Lb./acre of CMU	Crop and time of planting				
	Wheat, 1952	Oats, 1953	Wheat, 1953	Oats, 1954	Oats, 1955
1.6	49%	100%	100%	100%	100%
8	0	0	40	Partial*	100
16	0	0	Trace	Trace	65–70
32	0	0	0	0	Trace
64	0	0	0	0	0

* Owing to the very dry season reliable survival data were not obtained.

after the beginning of this experiment, since soil sampling had been discontinued, oats were sown across all of the plots. Survival data are shown in Table 4.

On the basis of the relatively small amount of data available, it is difficult to relate satisfactorily the point at which 100 per cent survival occurs to a definite CMU content in our soil. It could be pointed out that the CMU content of a plot which received 1.6 lb. per acre of CMU in 1952 was 0.13 lb. per acre in 1953 at which time oats showed 100 per cent survival. A plot which originally had received 16 lb. of CMU per acre and in June, 1954, showed a content of 0.80 lb. per acre was sown to oats that spring. Only the odd plant survived. On the other hand, a plot which in June, 1954, analysed 0.24 lb. of CMU per acre showed partial survival of the oats.

Control of Couch Grass by CMU

Since most of the experimental area was heavily infested with this weed observations with respect to its control by CMU were made, incidental to the main experiment. The couch grass was completely killed on those plots receiving over 16 lb. per acre of CMU, and an almost complete kill was achieved on the 16 lb. per acre plots. Examination of the 8 lb. per acre plots during the spring of 1953 indicated that most of the couch had been killed, but a small number of weakened plants remained, some of which subsequently died. It was felt that cultivation at that time would readily have killed the remaining plants. Figure 3 is a photograph illustrating the effect of CMU on couch grass in our experiment.

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The author wishes to express his appreciation to the Soils and Agricultural Engineering Departments, Ontario Agricultural College, Guelph, for supplying the land for the plots and carrying out all cultivation and seeding involved in this study. The co-operation of the Soils Department in providing precipitation data and soil data is also gratefully acknowledged.

FIGURE 3. Photograph illustrating couch grass control in the experimental area. One year after treatment.

Centre: 1.6 lb. per acre of CMU. A luxuriant stand of couch grass.
Left: 16 lb. per acre of CMU.
Right: 8 lb. per acre of CMU.

(Note the couch grass along the outer edges of the latter plots; some has been removed along the foreground edges to provide a better view of these plots.)

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FERTILITY STUDIES ON SOME NEW BRUNSWICK SOILS

I. SOIL PHOSPHORUS SUPPLY AS SHOWN BY GREENHOUSE AND CHEMICAL TESTS[1]

A. A. MacLean, J. J. Doyle and F. G. Hamlyn[2]

Experimental Farm, Canada Department of Agriculture, Fredericton, N.B.

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ABSTRACT

Fertility investigations have been conducted on soils collected from six farms on each of five soil types occurring in the Saint John River Valley. The effect of applied phosphorus on the yield and phosphorus content of ladino clover served as a basis for evaluating several chemical methods used in assessing the phosphorus status of different soils.

Applied phosphorus resulted in highly significant increases in yield and percentage of phosphorus in the crop on all soils. The degree of response, however, was not the same on all soil types. The higher the phosphorus content of the crop grown without phosphorus fertilizer, the lower was the increase in yield from applied phosphorus.

The soil phosphorus levels, as determined by seven of nine methods used, varied significantly between soil types. A positive correlation was found between soil phosphorus values, as obtained by six of the methods, and soil pH. The amounts of phosphorus extracted by two of the procedures employed decreased with increasing clay content.

Correlation coefficients relating soil phosphorus values and greenhouse results were highly significant for three of the nine methods employed.

INTRODUCTION

In the fall of 1952, an investigation was initiated to assess the phosphorus and potassium supplies in surface samples of a number of soil types in this province. One of the objectives in the study was to correlate chemical soil tests with crop response to applied fertilizer in greenhouse tests. The results pertaining to the phosphorus status of the soils are discussed in this paper.

A limited amount of work has been done on the relative value of different chemical methods for estimating phosphorus in Canadian soils (1), (5), (10). A report on the methods currently in use in the United States may be found in a recent U.S.D.A. publication (6).

MATERIALS AND METHODS

Composite samples of surface soil from six farms on each of five soil types were collected in the fall of 1952. Detailed descriptions of the soils may be found in soil survey reports (12), (13), (15). However, for convenience, the following brief description is given: (1) *Caribou loam*, a shallow brown loam to clay loam till, containing fragments of Silurian shale; (2) *Carleton loam*, a yellowish grey loam to clay loam till, containing fragments of Silurian shale and quartzite; (3) *Interval silt loam*, an immature alluvium subject to periodic flooding; (4) *Riverbank sandy loam*, a well drained water deposited sand; (5) *Tracy loam*, a red brown loam till containing fragments of red and grey coarse-grained sandstone.

[1] Presented before the Soils Section, Agricultural Institute of Canada, Macdonald College, June 22, 1954. Contribution from Experimental Farm, Canada Department of Agriculture, Fredericton, N.B.

[2] Research Officers and Technician, respectively.

The soils were air-dried, screened through a half-inch mesh and placed in glazed gallon pots on a weight basis. A sample of each soil was passed through a 2-mm. sieve and retained for chemical analyses. The fertility treatments consisted of phosphorus, potassium and lime applied singly and in combination in a 2 × 2 × 2 factorial design. In addition to the zero level, phosphorus (P_2O_5) and potassium (K_2O) were each applied at a rate of 200 lb. per acre and lime, in the form of calcium hydroxide, was applied as required to raise the soil pH to 6.5 as predetermined by titration curves (3). Ladino clover was planted in March, 1953, and, following germination, was thinned to two plants per pot. Fertilizer was applied at a depth of two inches and clover was seeded at a depth of one-half inch. The treatments were replicated three times. During the ensuing summer, four crops of clover were harvested. The phosphorus content of the crop, grown with and without fertilizer phosphorus, was determined on composite samples from all replications. Samples were ground in the Wiley mill and phosphorus was determined by the perchloric acid digestion method of Sherman (11).

The so-called available phosphorus contents of these soils were estimated by several chemical methods (2), (4), (7), (8), (9), (14). A summary of these is presented in Table 1. Phosphorus in the extracts was determined colorimetrically with a photoelectric colorimeter.

TABLE 1.—SUMMARY OF CHEMICAL METHODS

Procedure	Extractant	Ratio soil (gm.) to extractant (ml.)	Extraction time
Truog (14)	H_2SO_4 (0.002 N) buffered to pH 3.00	2 : 400	30 min.
Modified Truog (8)	H_2SO_4 (0.002 N) buffered to pH 3.00	4 : 400	30 min.
Modified Truog plus 8-hydroxyquinoline (4), (8)	H_2SO_4 (0.002 N) buffered to pH 3.00 + 8-hydroxyquinoline (0.1%)	4 : 400	30 min.
Peech and English (9)	NaAc + HAc (pH 4.85)	10 : 50	30 min.
Bray "adsorbed" (2)	NH_4F (0.03 N) + HCl (0.025 N)	1 : 7	60 sec.
Bray "adsorbed" plus "acid soluble" (2)	NH_4F (0.03 N) + HCl (0.1 N)	1 : 7	60 sec.*
Bray "acid soluble" (2)	By difference	—	—
HCl	HCl (0.1 N)	1 : 7	60 sec.
Olsen (7)	$NaHCO_3$ (pH 8.5)	5 : 100	30 min.

* Bray used an extraction time of 40 seconds.

RESULTS AND DISCUSSION

Greenhouse Results

The mean yields, the phosphorus contents of the clover and the amounts of phosphorus removed by the crop grown with and without fertilizer phosphorus are presented for each soil type in Table 2.

In this instance, as in all subsequent comparisons, values for the phosphorus treatment are means for all treatments which included phosphorus. Similarly, the no phosphorus treatment includes all treatments which did not receive the element. The results show that, under greenhouse conditions, applied phosphorus gave a highly significant increase in yield, in the percentage of phosphorus in the crop and in the amounts of phosphorus removed by the crop, on all soil types. The phosphorus content of the crop grown without applied phosphorus was somewhat higher on the Riverbank and Tracy soils and the response from applied phosphorus tended to be lower on these two soils. When the thirty soils

TABLE 2.—YIELD, PHOSPHORUS CONTENT AND AMOUNTS OF PHOSPHORUS REMOVED BY LADINO CLOVER

(Data are mean values per pot for six farms on each soil type; phosphorus applied at rate equivalent to 200 lb. P_2O_5 per acre)

Soil type	Yield		Phosphorus content		Phosphorus uptake	
	No phosphorus	Phosphorus	No phosphorus	Phosphorus	No phosphorus	Phosphorus
	gm.	gm.	%	%	mgm.	mgm.
Caribou loam	27.9	38.7	0.322	0.359	93.5	139.2
Carleton loam	25.1	35.6	0.301	0.346	80.1	124.0
Interval silt loam	28.5	39.3	0.316	0.367	92.6	144.6
Riverbank sandy loam	30.3	36.6	0.346	0.363	106.1	133.2
Tracy loam	27.4	35.5	0.332	0.355	90.8	126.8
L.S.D. (0.01)*	3.3		0.017		10.1	

* Based on pooled (treatment × farms) interaction within each soil type.

TABLE 3.—ANALYSES OF VARIANCE OF DATA RELATING TO YIELD AND PHOSPHORUS UPTAKE BY LADINO CLOVER

Source of variation	D.F.	Yield		Phosphorus uptake	
		M.S.	F.	M.S.	F.
Soil type	4	332.21	5.79**	2697.51	14.72**
Farms within soil type	25	633.69	11.05**	5717.09	31.19**
Phosphorus	1	15546.82	271.10**	100544.55	548.61**
Phosphorus × soil type	4	151.31	2.64†	4423.64	24.14**
Treatment × farms (error)	175	57.35	—	183.27	—

† Significant at 0.05.
** Significant at 0.01.

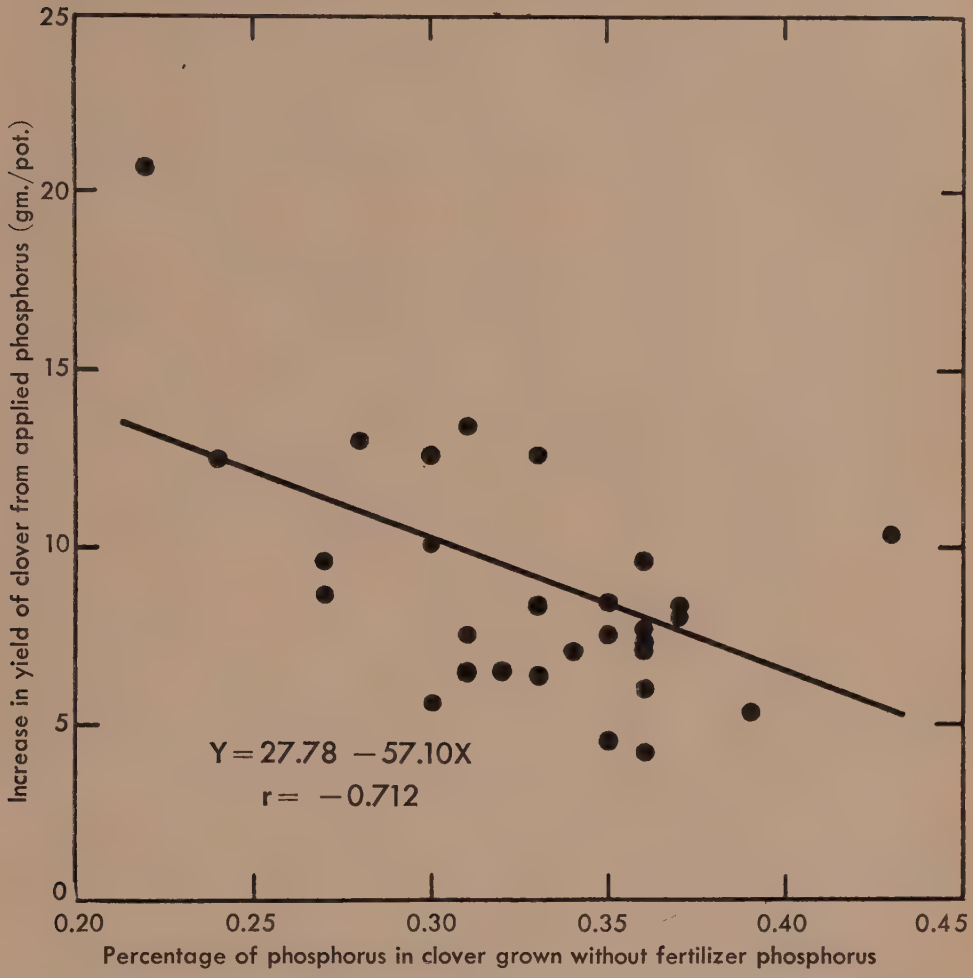

FIGURE 1. Relationship between phosphorus concentration of ladino clover and yield increase from applied phosphorus.

were considered, the higher the phosphorus content of the crop grown without applied phosphorus, the lower was the increase in yield from an application of this constituent (*Figure 1*). The correlation coefficient expressing this relationship (−0.712) was highly significant. These data indicate that plant analysis may be of value in diagnosing the phosphorus requirements of different soils.

The analyses of variance in Table 3 show that the variations in yield and in uptake of phosphorus by the crop between soil types and between farms within the same soil type were each highly significant. Although the effects of applied phosphorus on yield and uptake of phosphorus were highly significant, nevertheless, the interactions of phosphorus on soil types were significant. In other words, the effects of applied phosphorus on yield and on uptake of phosphorus by the crops were greater on some soil types than on others.

TABLE 4.—SOIL PHOSPHORUS VALUES AS DETERMINED BY VARIOUS CHEMICAL METHODS

(Data are mean values for 6 farms on each soil type)

Soil type	Truog	Truog (modified)	Truog (modified) plus 8-hydroxyquinoline	Peech and English	Bray "adsorbed"	Bray "adsorbed" plus "acid soluble"	Bray "acid soluble"	HCl	Olsen $NaHCO_3$-soluble
	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.
Caribou loam	24	23	24	2.2	26	38	12	15	19
Carleton loam	14	10	15	1.7	18	29	11	11	15
Interval silt loam	72	68	82	7.3	18	58	40	81	16
Riverbank sandy loam	26	26	29	3.5	50	87	37	22	28
Tracy loam	22	21	24	3.3	30	46	16	12	21
M.S. between soil type	3206.22	2974.97	3269.72	29.72	1008.95	3049.22	1194.12	5335.22	156.12
M.S. between farms within soil type	518.89	745.90	591.32	28.65	271.91	1085.85	333.00	1163.08	109.06
F value	6.18*	3.99*	7.39*	1.04	3.71*	2.81*	3.59*	4.59*	1.43

* Significant at 0.05.

Soil Phosphorus Extracted by Various Chemical Methods

The mean values for phosphorus as determined by different chemical methods are presented in Table 4. The variations in the amounts of phosphorus extracted from the different soil types by all methods, with the exception of Peech and English and Olson's sodium bicarbonate, exceeded the corresponding variations between farms on the same soil type at the 5 per cent level.

With this significant variation occurring in the amounts of phosphorus extracted from different soils, it appeared desirable to determine if such variability were associated with any particular soil property. The correlation coefficients relating soil phosphorus and soil pH values were significant for six methods at the 1 per cent level. The coefficients were +0.517 for Truog, +0.549 for modified Truog, +0.500 for modified Truog plus 8-hydroxyquinoline, +0.487 for Bray "acid soluble", +0.577 for HCl and +0.527 for Peech and English. The Bray "adsorbed" and Bray "adsorbed" plus "acid soluble" values were negatively correlated with the clay content of the soils. The coefficients were −0.497 and −0.404, respectively, and were significant at the 5 per cent level.

Relationship Between Soil Phosphorus Values and Crop Response in the Greenhouse

In the evaluation of chemical methods a number of criteria might be employed as measurements of crop performance. In this instance, total phosphorus uptake was used as it embraced both yield and phosphorus content of the crop. The uptake of the element by the crop on the pots receiving no phosphorus was expressed as a percentage of the uptake on those receiving phosphorus. The correlation coefficients, relating soil phosphorus values and phosphorus uptake, which were calculated without regard to soil type, are presented in Table 5. The results show that some

TABLE 5.—RELATIONSHIP BETWEEN SOIL PHOSPHORUS VALUES AND PHOSPHORUS UPTAKE BY LADINO CLOVER

(Uptake on no-P treatment expressed as percentage of uptake on P treatment)

Chemical method	D.F.	Correlation coefficient
Truog	28	+0.237
Truog (modified)	28	+0.285
Truog (modified) plus 8-hydroxyquinoline	28	+0.201
Peech and English	28	+0.289
Bray "adsorbed"	28	+0.732**
Bray "adsorbed" plus "acid soluble"	28	+0.650**
Bray "acid soluble"	28	+0.461*
HCl	28	+0.181
Olsen	28	+0.727**

* Significant at 0.05.
** Significant at 0.01.

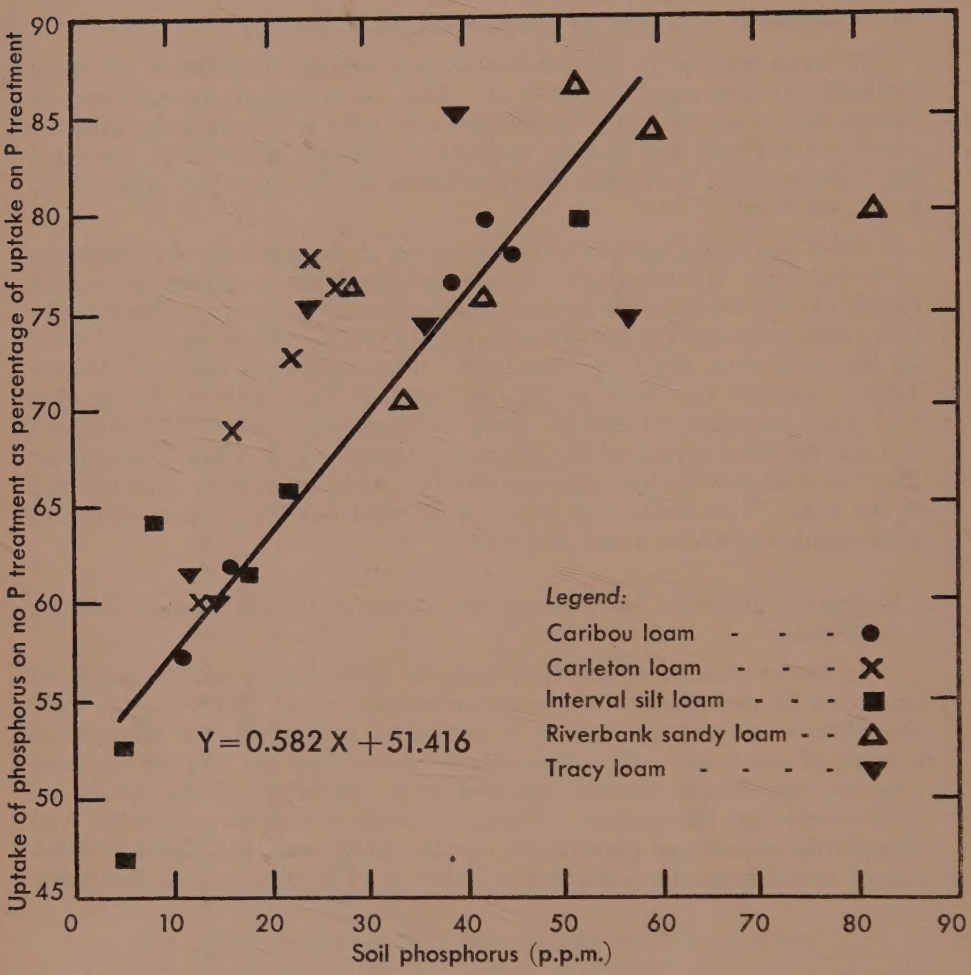
Uptake of phosphorus on no P treatment as percentage of uptake on P treatment
Soil phosphorus (p.p.m.)
Y = 0.582 X + 51.416
Legend:
Caribou loam
Carleton loam
Interval silt loam
Riverbank sandy loam
Tracy loam

FIGURE 2. Relationship between Bray "adsorbed" phosphorus and crop response.

TABLE 6.—RELATIONSHIP BETWEEN SOIL PHOSPHORUS VALUES AND PHOSPHORUS UPTAKE ON INDIVIDUAL SOIL TYPES

(Uptake on no-P treatment expressed as percentage of uptake on P treatment)

Soil type	D.F.	Correlation coefficients				
		Bray "adsorbed"	Bray "adsorbed" plus "acid soluble"	Olsen $NaHCO_3$-soluble	Peech and English	Truog
Caribou loam	4	+0.931**	+0.921**	+0.910*	+0.819*	+0.867*
Carleton loam	4	+0.903*	+0.892*	+0.819*	+0.648	+0.722
Interval silt loam	4	+0.892*	+0.890*	+0.889*	+0.802	+0.859*
Riverbank sandy loam	4	+0.526	+0.569	+0.337	+0.411	+0.628
Tracy loam	4	+0.702	+0.595	+0.658	+0.601	+0.576

* Significant at 0.05. ** Significant at 0.01.

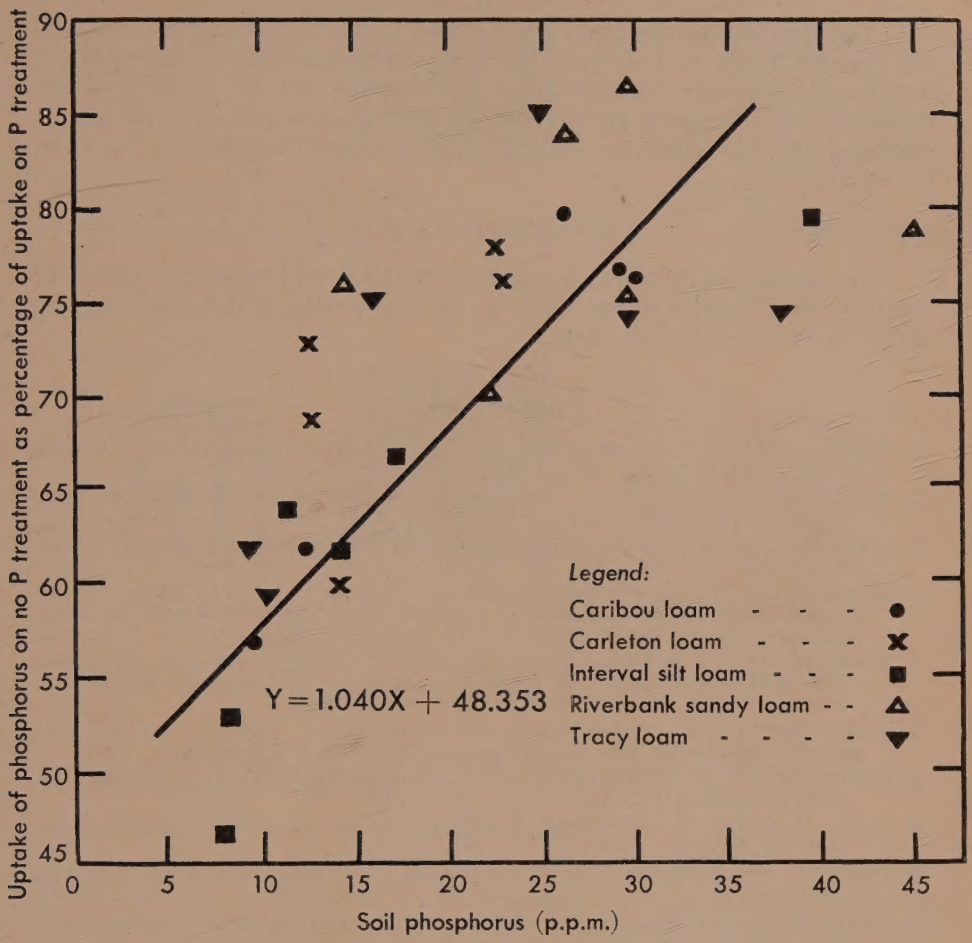

FIGURE 3. Relationship between $NaHCO_3$-soluble phosphorus and crop response.

methods were more satisfactory than others and that the methods of Bray for "adsorbed" and "adsorbed" plus "acid soluble" phosphorus and of Olsen for $NaHCO_3$-soluble phosphorus were the most satisfactory for the soils investigated.

Six phosphorus values as determined by a particular method were available for each soil type. Accordingly, it was possible to relate the soil phosphorus values and greenhouse results on each soil type. The results reported in Table 6 indicate that the chemical methods were more satisfactory for some soils than for others. No significant correlation was obtained with any method on Riverbank sandy loam and Tracy loam soils. Observations of the response pattern on these soils showed that the six farms sampled tended to be more similar than in the case of other soil types. Accordingly, it might be expected that it would be more difficult to obtain a significant correlation on the basis of small differences.

The relationship between phosphorus uptake by the crop and soil phosphorus levels as indicated by Bray "adsorbed" and Olsen sodium bicarbonate methods are illustrated in Figures 2 and 3. In spite of the relatively high degree of correlation obtained in each instance, it may be observed that the values for individual soils deviate considerably from the best fitting line.

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